

**Development of *Couroupita guianensis* Incorporated Edible Films for
Food preservation**

**Kaviyarasi, P
(15PBC006)**

**A Thesis Submitted to
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore-641043**

**In Partial Fulfilment of the Requirement of the Degree of
Master of Science in Biochemistry**

April, 2017

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Signature of Head of the Department


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Signature of Supervisor

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1.0 INTRODUCTION

Food safety is currently a global concern as large number of people around the world is facing intense problems due to consumption of spoiled foods. People gain more information and become aware about the food safety issues through the availability of various sources such as the internet and social media (Pinu, 2016).

In people's daily diet, vegetables are one of the most important food supplies that can provide a variety of vitamins and minerals due to which the determination of the natural preservatives in vegetables has gained a very significant importance (Nielsen *et al.*, 2017).

Microorganisms breeding or microbial contamination is one of the main causes for spoilage of foods. Foods are commonly spoiled by the growth of gram positive and gram negative bacteria which thrive well on fruits and vegetables. The spoilage is considered to be the bad fermentation process which leads to accumulation of acids in the spoiled foods. Thus, the spoilage of foods results in loss of total food supply (Moretro *et al*, 2016).

Different types of *Salmonella* as well as *Escherichia coli* have been implicated in food borne outbreaks by consumption of cantaloupe, honey dew, and watermelon (CDC 2007).The optimum extension of the post harvested life of food products is critically dependent upon three factors: reduction in desiccation, reduction in physiological process of maturation and senescence and reduction in the onset and rate of microbial growth (Dhall, 2013).

The Center for Disease Control and Prevention has estimated that 48 million citizens in the world become ill from ingesting food borne pathogens every year; and roughly one third of foods globally go to waste due to spoilage (Food and Agricultural Organization United Nations, 2011). These numbers underline that much can still be done to improve food preservation.

Now-a-days, the industries have been using the indicator microorganisms for determining the microbial quality and status hygiene level for their products. The loss of massive amount of the food products can be controlled by an early detection of spoilage microorganisms and it is an important step to overcome the outbreaks of the spoiled foods (Siroli *et al.*, 2015).

The addition of preservatives and the use of the antimicrobial agents which can also be directly incorporated into the food, increase the shelf life of the products. Food additives are a mixture of substances or any substance which is added to the food in minimal quantity other than basic food components. Such antimicrobial additives maintain the nutritional value of foods and find their importance in food industry. The use of the efficient and low toxic preservatives maintains the flavor of vegetables year around sales (Cai *et al.*, 2015).

Chemical preservation is one of the important practices in modern food technology to gain faster yield with increase in the production of processed and convenience foods. Such chemicals bring about nutritional losses and cause enzymatic or chemical changes in the concerned food. Still chemical preservatives are considered to be the foolproof for preservation processes. But, when excess amount of the chemically preserved foods are consumed, it leads to toxicity. Some commonly used preservatives are Butylated hydroxyl anisol (BHA), Butylated hydroxyl toluene (BHT), Sodium nitrate, Propyl gallate and Sorbic acid (Mathenjwa *et al.*, 2012).

Due to the potential toxicity of chemical preservatives, there has been increased demand for food preservatives from natural sources. This has led researchers and food processors to come across natural food additives with a wide range of antimicrobial activities (Pandey *et al.*, 2014).

There is a wide range of the techniques used to extend the shelf life of foods, using edible coatings. Preservation or developmental systems such as

packaging, films, and coatings, is an on-going area of the study. Edible films and coatings have recently gained more interest in the field of food preservation due to the promising results obtained and have been recently reviewed. Edible coating is a thin layer of edible material which can be coated on food it can be applied to the food either in solid or liquid form. Solid sheets are used to wrap food products. Compared to other processes of food preservation, the edible films and coatings improve the structural integrity and mechanical properties of foods and also act as a barrier against the water vapor and gases (Jabeen *et al.*, 2014)

As a result, plant antimicrobial products have acquired importance in food system to retard bacterial and fungal growth. Consumer interest is also increasing in consumption of food products having natural ingredients. Thus, natural extracts with more of biologically active substances are able to influence the safety and quality of foods, posing as better alternatives to synthetic additives (Prakash *et al.*, 2015)

One of the more fashionable trends consists of the development of innovative biopolymers obtained from agricultural commodities and or food-waste products. The use of biopolymers in multiple food-packaging applications has emerged as an alternative with regard to their film-forming properties to produce edible films as an environmentally friendly technology (Saller *et al.*, 2017).

Starches, cellulose derivatives, chitosan, gums, animal or plant-based proteins, and lipids offer one the possibility of obtaining edible films in fresh or processed food packaging to extend the food's shelf life. These polymers offer additional advantages in their commercial use, such as biocompatibility, barrier properties to moisture and/or gases, no toxicity, nonpolluting characteristics, mechanical integrity, and low cost. In addition, edible films can act as carriers for antioxidant/antimicrobial additives to extend food's shelf life while maintaining their mechanical integrity and handling characteristics.

Antioxidant edible films can prevent food oxidation, the development of off-flavors, and nutritional losses, whereas antimicrobials can prevent spoilage from food-borne bacteria and organoleptic deterioration by microorganism proliferation (Shah *et al.*, 2015)

Starch films are edible, the chemicals are not suitable. Consumer prefers modified starch based films, this way starch modifiers have been developed. Several plant extract has been used to combat spoilage in foods.

Natural plant derived compounds can be obtained from fruits and vegetables (Garlic, pepper, onion, cabbage, xocconostle, and guava), seeds and leaves (olive leaves, Parsley, caraway, nutmeg, fennel and grape seeds), and herbs and spices (marjoram, basil, oregano, rosemary, thyme, sage, clove and cardamom). Plant derived essential oils and extracts have long been used as a food additive not only to enhance taste and impart characteristics flavors but also used to prolong food shelf life by preventing rancidity and controlling microbial contamination. In fact, due to their high content of secondary metabolites mainly phenolic compounds such as iso flavanoids, terpenes, ketones, aliphatic alcohols, acids, aldehydes, and these compounds are able to reduce or inhibit the growth of the pathogenic microorganisms (Aloui and Khwaldia, 2016).

One such plant is the *Couroupita guianensis* which belongs to the family Lecythidaceae. It is chiefly tropical and subtropical in distribution. *Couroupita guianensis* Aubl (Cannon Ball tree) is a deciduous, tropical tree growing to 35 m high, the tree is widely found all over the world and has enormous medicinal values. Most of the parts like leaves, flowers, fruit, and stem are used as medicine to cure various diseases (Sivakumar *et al.*, 2012)

Earlier studies on antimicrobial potential of the plant *Couroupita guianensis* have been carried out in our laboratory which involved the

identification of several phytochemical compounds in various parts of the plant. These parts include the leaves, flowers, fruit and bark of the plant.

The antimicrobial activity of the leaves of *Couroupita guianensis* was discerned by using extracts of the leaves from various solvents such as methanol, aqueous and chloroform. Among the extract studies, methanol extracts had a better antimicrobial activity comparatively. Also, among the various bacteria tested for their susceptibility to the methanolic extract of *Couroupita guianensis*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were found to be most susceptible. Hence, *Klebsiella pneumoniae* and *Staphylococcus aureus* were chosen for the present study and was aimed with the following objectives.

- To form a good biodegradable edible film incorporated with *Couroupita guianensis* extract.
- To discern the inhibitory capacity of the incorporated edible film against *Klebsiella pneumoniae* and *Staphylococcus aureus* organisms.
- To standardize the effective inhibitory concentration of extracts in the edible films.
- To confirm the efficacy of the developed edible film using *in vitro* model system.

2.0 REVIEW OF LITERATURE

The review of literature for the present study entitled Development of *Couroupita guianensis* incorporated edible films for food preservation has been discussed under the following headings.

2.1 Food Spoilage

2.2 Chemical Preservatives of Food

2.3 Historical view of Edible Films

2.4 Composition of Edible Films

2.5 Properties of Edible Coatings

2.6 Fruits and Vegetables that are to be Coated

2.7 Edible Biofilm

2.8 Plant as Preservatives

2.9 *Couroupita guianensis*

2.9.1 Traditional Uses

2.9.2 Antibacterial Activity of *Couroupita guianensis*

2.1 FOOD SPOILAGE

Food and food spoilage moulds cause great economic losses worldwide. It is estimated that between 5 and 10% of the world's food production is wasted due to bacterial and fungal deterioration. These fungi cause losses in dry matter or quality and some species can produce health damaging mycotoxins. Many mycotoxins are stable under normal food processing conditions and therefore be present not only in food and feed but also in processed products (Amensour *et al.*, 2010).

Food contamination and spoilage by microorganisms is a unresolved problem, despite the range of preservation techniques available, the microbiological safety of food continues to be a major concern to consumers, regulatory agencies and food industries throughout the world. Microbial contamination still poses important public health and economic concerns for the human society. Among many strategies to inhibit the growth of undesirable microorganisms, is the use of chemical agents that exhibit antimicrobial activity. These chemicals may be either synthetic compounds intentionally added to foods or naturally occurring and biologically derived substances. However current consumer demand for more natural and fresh-like foods containing fewer synthetic additives but increased safety and shelf life, have encouraged food manufacturers to use natural or mild preservation techniques, with the results that alternative sources of safe, effective and acceptable natural preservatives need to be explored (Floros,2010) .

2.2 CHEMICAL PRESERVATIVES OF FOOD

Preservation of food stuffs have always been a necessity of reasons the durability of food is limited numerous food stuffs are only available during a

short harvesting season and the transport routes of food or raw materials from the production site to the consumers are continuously increasing in length. There are several medical hygienic efforts aimed at inhibiting growth of microorganisms in food. The hazard to health which many bacteria carry has been long known. Recently, a number of fungi have been shown to form toxins during their growth on food stuffs. The preservatives now in use have been thoroughly tested for their toxicological properties but still their use in the food industry is subject to stringent legal regulations (Bouwmeester *et al.*, 2009).

Mostly the chemical preservatives are used in the fruit juices and in the beverages mainly to increase their shelf life. Because of such concern, efforts are taken to develop potentially effective, healthy, and natural food preservatives. The utilization of plant extracts as antimicrobial agents for the preservation of the food is considered to be nutritionally safer and easily degradable (Bouwmeester *et al.*, 2007).

2.3 HISTORICAL VIEW OF EDIBLE COATINGS

Edible coatings are thin layers of edible material applied to the product surface in addition to or as a replacement for natural protective waxy coatings and to provide a barrier to moisture, oxygen, and solute movement for the food. They are applied directly on the food surface by dipping, spraying, or brushing to create a modified atmosphere. Because they will be consumed, the material used for the preparation of edible films and coatings should be generally regarded as safe (GRAS) approved by FDA and must conform to the regulations that apply to the food product concerned. An ideal coating is defined as one that can extend storage life of fresh fruits and vegetables without causing anaerobiosis and reduces decay without affecting their quality. Previously, edible coatings have been used to reduce water loss, but recent developments of formulated edible coatings with a wider range of permeability characteristics has extended the potential for fresh produce application (Bourtoom, 2008).

Edible and biodegradable coatings must meet a number of special functional requirements, for example, moisture barrier, solute or gas barrier, water/lipid solubility, color and appearance, mechanical characteristics, no toxicity, etc. The effect of coatings on fruits and vegetables depends greatly on temperature, alkalinity, thickness and type of coating, and the variety and condition of fruit and vegetable. The functional characteristics required for the coating depend on the product matrix (low to high moisture content) and deterioration process to which the product is subjected. The use of wax coating of fruits by dipping is one of the age-old methods that were in vogue in the early 12th century. This was practiced in China, essentially to retard water transpiration loss in lemons and oranges (Mellinas *et al.*, 2016).

Later fat coating on food products, specifically called “larding” was in vogue in England. “Sausage casing” used very commonly nowadays is nothing but a material derived from a protein source (gelatin). Usually a film thickness of 2.5 mm is employed and coating is done by several methods. Observed that oils, waxes, or cellulose had similar effects of preventing spoilage and retaining fresh-picked quality of fruits and vegetables. Several attempts have been made to develop other materials that could be used to coat, produce, and modify internal gas composition of fruit/vegetables for short-term storage (Baldwin *et al.*, 1995).

In 1982, Lowings and Cutts reported an edible coating material that is nonphytotoxic, tasteless, odorless, and is effective in increasing shelf life of fruits and vegetables. This coating material is a mixture of sucrose fatty acid esters (SFAE), sodium carboxymethyl cellulose, and mono- and diglycerides. SFAE was originally developed as an emulsifier. However, coating of SFAE on fruits retards fruits’ ripening. SFAE mixtures have been commercially available since 1980’s for coating fruits and vegetables under the trade names “TAL Pro-long” and “Semper fresh” reported that application of zein coating

on the tomato surface delayed its color change, weight loss, and maintained firmness during storage (Dhall, 2013).

2.4 COMPOSITION OF EDIBLE COATINGS

Edible coatings can be produced from materials with film forming ability. During manufacturing, film materials must be dispersed and dissolved in a solvent such as water, alcohol, mixture of water and alcohol, or a mixture of other solvents. Plasticizers, antimicrobial agents, minerals, vitamins, colors, or flavors can be added in this process, adjusting the pH and/or heating the solutions may be done for the specific polymers facilitate dispersion. Film solution is then casted and dried at a desired temperature and relative humidity to obtain freestanding films (Ortega *et al.*, 2016)

Edible coatings may be composed of polysaccharides, proteins, lipids, and composites. Their presence and abundance determine the barrier properties of material with regard to water vapor, oxygen, carbon dioxide, and lipid transfer in food systems. However, none of the three constituents can provide the needed protection by themselves and so are usually used in a combination for best results. Edible coatings can be made from a variety of polysaccharides. However, polysaccharides, being hydrophilic in nature, do not function well as physical moisture barriers. The method by which they retard moisture loss is by acting as a sacrificial moisture barrier to the atmosphere, so that the moisture content of the coated food can be maintained. In addition to preventing moisture loss, some types of polysaccharide films are less permeable to oxygen, which can help to preserve certain foods (Campos *et al.*, 2010).

On the other hand, these polysaccharides make materials with excellent gas barrier property. The principal polysaccharides of interest for material production have been cellulose, starch, gums, and chitosan. Linear structure of some of these polysaccharides, for example, cellulose, amylose (a component

of starch), and chitosan renders their films tough, flexible, transparent, and resistant to fats and oils. Likely, the more complex polysaccharides produced by fungi and bacteria such as xanthan, curdlan, pullan and hyaluronic acid will receive more interest in the future (Azeredo and Waldron, 2016).

2.5 PROPERTIES OF EDIBLE COATINGS

The properties of edible coating depend primarily on molecular structure rather than molecular size and chemical constitution. Specific requirements for edible films and coatings are the coating should be water-resistant so that it remains intact and covers a product adequately, when applied. It should not deplete oxygen or build up excessive carbon dioxide. A minimum of 1–3% oxygen is required around a commodity to avoid a shift from aerobic to anaerobic respiration (Clarke *et al.*, 2016).The important properties of edible coatings include the4 following :

- It should reduce water vapor permeability.
- It should improve appearance, maintain structural integrity.
- Improve mechanical handling properties, carry active agents (Antioxidants, vitamins, etc.) and retain volatile flavor compounds.
- It should melt above 40°C without decomposition.
- It should be easily emulsifiable, non-sticky or should not be tacky, and have efficient drying performance.
- It should never interfere with the quality of fresh fruit or vegetable and not impart undesirable order.
- It should have low viscosity and be economical.
- It should be translucent to opaque but not like glass and capable to tolerate slight pressure.

2.6 FRUITS AND VEGETABLES THAT ARE TO BE COATED

It is important to note that edible coatings are not used all the time on any fruit or vegetable. It can be applied on whole and fresh-cut fruits and vegetables. Fruits and vegetables that are coated as whole include apple, kinnow, grapefruit, passion fruit, avocado, orange, lime, peach, lemon. Vegetables that are used for coating as whole are cucumber, bell pepper, melons, tomato (Olivas and Canovas., 2005).

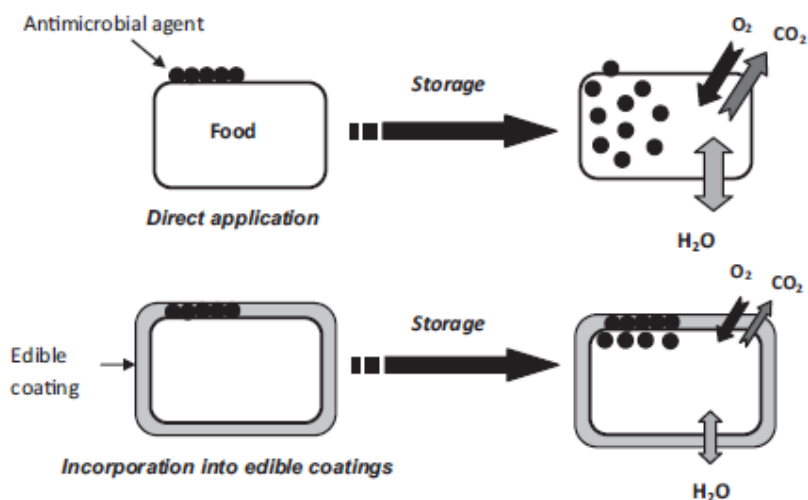
Fresh-cut products are highly perishable, the main reason being removal of skin (the natural protective layer) from their surface area and the physical stress they undergo while peeling, cutting, slicing, shredding, trimming, coring, etc. Fresh cut fruits and vegetables on which coating is used commercially. They are fresh cut apple, fresh cut pear, and fresh cut peach. Minimally processed carrot, fresh cut lettuce, fresh cut cabbage, minimally processed onion, fresh cut potato, fresh cut tomato slices, fresh cut muskmelon and cantaloupe are used for coatings (Gousia *et al.*, 2013).

2.7 EDIBLE BIOFILM

Edible coatings are produced from different natural components; the quality mainly depends on barrier property, chemical composition, structure of the coating materials and storage conditions. It reduces gas exchange between the food products and the environment. The experiments were done on grapes, papaya, mango and strawberries. Recently, the use of edible coatings for the entrapment of the natural antimicrobial agents has been investigated as a promising alternative. Edible coating acts as a semi permeable barrier and also protects the additional protection for foods. It is a thin layer prepared from

naturally occurring polymers by different procedures such as spraying, brushing and dipping (Galus and Kadzinska, 2015).

The functional properties of the edible coatings are viscosity of the coating solutions, coating thickness, degree of cross linking and the concentration of the additives include (emulsifiers, plasticizers or cross linking agents). The performance of edible coatings was improved by the incorporation of different bioactive substances or compounds, mainly the antimicrobial agents, not only increase antimicrobial properties but also reduce the biochemical deteriorations. The antimicrobial delivery systems are mainly to extend the shelf life of highly decomposable food stuff, mainly fresh and minimally processed fruits (Olivas and Canovas, 2005).



The polysaccharide such as the sodium alginate have been used as coating materials for fresh fruits and vegetables because of their excellent film forming property and permeability of the oxygen and the carbon dioxide. The accumulation of carbon dioxide and oxygen in the internal atmosphere may lead to anaerobic fermentation. The effectiveness of preserving the fresh fruits and vegetables are based on the quality of the coating formulations. The environmental conditions, storage area, coating permeability, and the respiration affect the internal atmosphere of the fruits and the vegetables (Shah *et al.*, 2013).

Many researchers have been evaluating the efficacy of these antimicrobial agents in edible films and coatings. Several *in vitro* studies have been performed to assess the antimicrobial activity of the film forming solutions incorporated with the natural antimicrobial agents, main advantage in the film is that can be consumed with the packaged products. No package to dispose, even the films are not consumed they could contribute to the reduction in the environmental pollution. Films are produced from renewable and from edible ingredients and degrade more readily than other materials. The films can be applied inside the foods at the interfaces, to prevent deteriorative inter component moisture and solute migration in food (Fai *et al.*, 2016).

During manufacturing process, the film material should be dissolved in the solvents such as the alcohol or water or in the mixture of other solvents and plasticizers, antimicrobial agents, colors or flavors can be added to it. The heating is done to facilitate the dispersion. Film solution is then casted and dried at a desired temperature to obtain free standing films. In the food industry, it is applied by several methods. The films can be prepared based on three categories such as hydrocolloids, lipids and composites (Salleh *et al.*, 2014).

The polysaccharides have a poor water vapor and gas barrier properties, these coating act as a sacrificing agents to retard the moisture loss from the food products. Starch is a polymeric carbohydrate composed of glucose units. So it is often used in the industrial foods, which produce biodegradable films too partially or entirely at low cost. Normally the corn starch consists of approximately 25%amylase and 75%amylopectin the film which is produced from corn starch had no detectable oxygen permeability both in the plasticized and unplasticized films. Thus the addition of the plasticizers to the film leads to increased gas permeability. Partial etherification of the starch improves the water solubility (Basiak *et al.*, 2017).

2.8 PLANTS AS PRESERVATIVES

The preservatives are natural or manmade chemicals that are added to foods to stop them from spoiling. We actually preserve fresh foods ourselves everyday by storing foods in the fridge or freezer. Natural preservatives that can be added to foods include Sugar, Rosemary extract, Lemon juice, Salt. Natural preservatives are those that help keep foods fresh looking and tasting longer and prevent them from spoiling (Giner *et al.*, 2012).

Artificial food preservatives are responsible for causing many health problems pertaining to respiratory tract, heart, blood and other. But, besides this there is no major health concern associated with the natural food preservatives. Natural preservatives are absolutely safe for consumption. It does not alter the color of the food and gives the required flavor (Bourdichon *et al.*, 2012).

Plant contain more of antimicrobial agents, screening of those antimicrobial agents gained much more importance for the development of quality and nutritional rich foods. The side effects caused by the use of the chemical preservatives and synthetic antimicrobial agents have been paved the way for potentially active agents, from the natural sources, such as from the plant and microbial sources, example such as rosemary extract is also a prolonged standing natural preservative which is obtained from distillation of rosemary leaves. These have antioxidant qualities which make them excellent for extending the shelf life of food stuffs. Grape extracts which is prepared from grape seeds, pores, skin and stems, has important antibacterial activity which can be employed for food stuff (Yuan *et al.*, 2016).

Some food additives as *Origanum vulgare* have an important inhibitory effect on *Aspergillus*, providing an inhibition of all assayed strains. Food preservation techniques are used to provide food with high nutritional quality and microbial stability, which is achieved by controlling the growth or survival of spoilage associated and food borne microorganisms. Historically several physical or chemical procedures have been used to ensure the microbiological safety of the foods (Martelli *et al.*, 2017).

More than 1,340 plants are known as potential source of antimicrobial compounds, but very few of these have been studied scientifically. The scenario has encouraged research aiming at the possible development and use of the vegetable products with antimicrobial properties, studies conducted in certain species have demonstrated several pharmacological properties of plants within the genus, such as antidiabetic, anti-inflammatory, antiulcerogenic, analgesic and antihypertensive activities (Iturriaga *et al.*, 2012).

The side effects caused by the use of the chemical preservatives and synthetic antimicrobial agents have been paved the way for potentially active agents, from the natural sources, such as from the plant and microbial sources (Gonclaves *et al.*, 2017).

2.9 COUROUPITA GUIANENSIS

Scientific name: *Couroupita guianensis*

Common names:

English: Cannon ball tree

Hindi : Shivaling Ayahuma

Spanish: Coco de mono

BOTANICAL CLASSIFICATION:

Kingdom	: Plantae -Plants
Sub kingdom	: Tracheobionta-vascular plant
Super division	: Spermatophyta-seed plants
Division	: Magnoliophyta –Flowering plants
Class	: Mangoliopsida-Dicotyledons
Subclass	: Dilleniidae
Order	: Lecythidaceae-Brazil –nut family
Family	: <i>Couropita</i> Aubl-Cannon ball tree
Species	: <i>Couropita guianensis</i> Aubl-Cannon ball tree

Medicinal plants are used as an ancient style of providing cure to many ailments. *Couropita guianensis* is a vital healthful plant in Chinese medication which is supposed to have varied medicinal activities like antipyretic, antidepressant, antiseptic, analgesic, antioxidant and antifertility activities. Several different constituents such as steroids, glycosides, carbohydrates, couroupitone A, couroupitone B, isatin, eugenol, linoleic acid, nerol, tryptanthrin, linalool, phenolic resin substances and dyes are found to be present in *Couropita guianensis* (Swapnalatha and Rajeshwari , 2014).

Couropita guianensis belongs to family Lecythidaceae, could be a massive deciduous tropical tree 90'tall. It's fully grown in Indian garden as a decorative tree for its enticing flowers. It's remarkable referred to as 'Canon ball tree' in English "Kailaspat" in Hindi. In recent times there are several reports of medicinal roles and activities of *Couropita guianensis* and its active principles on the nervous system and antipyretic immunomodulatory (Kavitha *et al.*,2011).



PLATE 1: *Couroupita Guianensis*

Cannon ball tree is a deciduous tree native to tropical South America; it has large, apricot pink and gold flowers with an unusual, lopsided arrangement of central stamens and a penetrating fragrance .It is really a wonderful tree doesn't grow branches that reach out from the straight trunk, it bears vast showy flowers with 3''to 5''waxy aromatic smelling growing directly on the bark of the trunk.

The tree additionally produces orbicular brown, woody, indehiscent double fleshy fruits of associate degree astonishing size, adequate to the scale of an individual head. The fruit includes of little seeds in an exceedingly white, unpleasant smelling edible jelly size of a mature fruit is 1450gms and weight of the shell from a fruit is 545gms. It's wide planted in tropical and subtropical biology gardens as a decorative, throughout the tropics and sub tropics (Gousia *et al.*, 2013).

2.9.1 TRADITIONAL USES:

The pulp of the fruit of the cannon ball tree is rubbed on the infected skin of animal disease. Traditionally the leaves are used as antiseptic and odontalgia. Juice made up of the leaves is used as ointments to cure skin ailments and shamans of South America have been used the tree components for treating protozoan infection.

Historically the leaves of this plant are utilized in the treatment of skin diseases, stomach ache and internal gas formation, antithrombotic and vasodilator actions. Leaves and the flowers of *Couroupita guianensis* used for applications like upset tumors, pain and inflammatory processes, cold, enteric gas formation and abdomen ache. The volatile oils from the flowers show antibacterial and antifungal properties. It's one in every ingredient within the several preparations that cure redness, hemorrhage, piles, scabies, dysentery and scorpion poison (Manimegalai *et al.*, 2014)

2.9.2 ANTIBACTERIAL ACTIVITY OF *Couroupita guianensis*:

The antibacterial activity in methanol extract of *Couroupita guianensis* fruit pulp by maceration and it was studied against Gram positive and Gram negative bacteria. Compared to Ciprofloxacin and fluconazole, vital activity was found against *Bacillus subtilis* at concentration 4mg as compared to further tested organisms. This study explains the presence of some phytoconstituents like tannins, sugars, and polyphenols (Majumder *et al.*, 2014).

The zone of inhibition by chloroform extract ranged from zero to twenty six millimeter. Chloroform extract showed effective antimicrobial activity against the gram negative organisms. The results of the antimicrobial activity showed effective repressing activity against *Plesimonas shigelloides*, *Cocci aureus*, and *Proteus vulgaris*. Moderate antimicrobial activity was recorded against *E. coli*, *Klebsiella pneumoniae* and *Salmonella typhi* (Lee and Min, 2013).

3.0 METHODOLOGY

Edible coatings are an environmentally friendly technology that is applied on many products to control moisture transfer, gas exchange or oxidation processes. Application of antimicrobial agents directly on the food surface may have limited benefits, because the active substances are rapidly neutralized or diffused, from the surface into the food product, thus limiting the effect of the antimicrobial compound. In this sense, antimicrobial incorporated edible films and coatings may provide increased inhibitory effects against spoilage and pathogenic bacteria by maintaining effective concentrations of the active compounds on the food surfaces.

Many factors must be considered in developing an antimicrobial edible coating, which includes the properties of the food and coating and the effectiveness of the antimicrobial agents incorporated into the coating. Because of this, basic preliminary studies must be carried out to evaluate the antimicrobial effect of a compound incorporated into an edible film matrix before it is applied on the surface of a real food system.

Since natural antimicrobials are more preferred, plant extracts serve as valuable sources for incorporating in films. *Couroupita guianensis* is a tree which possesses antibiotic, antifungal and analgesic qualities. Chemistry of this species has showed the presence of the nerol, tryptanthrine, indigo, indirubin, linoleic acid, carotenoids and sterols. The trees are used to cure cold and stomach aches. The earlier studies about the *Couroupita guianensis* have shown the presence of antibacterial activity against certain pathogens.

Couroupita guianensis has been chosen for the present study because of its wide spectrum of antibacterial potential. This antibacterial potential of the selected plant parts namely leaves, bark, flowers have already been proven in

our laboratory. Among all the solvents used for extraction, the methanolic extracts from the flowers were found to have the maximum antimicrobial activity. Comparing among the broad spectrum of bacteria, *Staphylococcus aureus* and *Klebsiella pneumoniae* were found to be more susceptible.

Hence, in the present study, the methanolic extract of the flowers were selected and checked for its preservative efficiency against *Staphylococcus aureus* and *Klebsiella pneumoniae* by incorporating in a starch based biopolymer.

3.1 PREPARATION OF EDIBLE FILMS

The tendency to use natural and renewable polymers in various fields is growing so that they can replace synthetic packaging films. In this regard, the starch is well known as a green material. This material is biodegradable, edible, and is not dependent on fossil resources while it is extensively available.

The starch solutions required for the preparation of edible films were obtained by dispersing 3 or 5 g of corn starch in 100 ml of distilled water. After complete dispersion, 100g/kg (for each gram of starch, 0.1 g of plasticizer was added) of plasticizer was added.

The filmogenic gelatin solution was obtained by the hydration of 10g of gel in 100ml of the distilled water for one hour at room temperature. After this period the solution was heated at 70°C and maintained at this temperature for 10 min. The plasticizer (sorbitol or glycerol) (100 g/kg of the gel) was then added with constant gentle stirring to prevent air bubbles and gelatin denaturation.

In order to obtain edible films, the starch and gel solutions were mixed in a ratio of 1:1 (50 weight percentage of starch and 50 weight percentage of gel). Aliquots of 20 ml of the resulting solution were added in petridishes. The films were then dried at room temperature (25° C) for 24 hours and stored.

3.1.1 INCORPORATION OF *Couroupita guianensis* EXTRACT IN EDIBLE FILMS

The methanolic extracts of the flowers of *Couroupita guianensis* at various concentrations (50mg, 75 mg, 100 mg, 125 mg, 150 mg) were added to the starch and gel solutions, which were prepared during the development of edible films. The *Couroupita guianensis* incorporated films obtained from this procedure were used for the subsequent tests.

3.1.2 Analysis of water and acid solubility:

In order to determine the bioplastic solubility in water and acid, the *Couroupita guianensis* incorporated films developed were subject to water and acid solubility analysis. This was performed to give an idea of the solubility of the developed incorporated edible film when the coated vegetables are consumed. Since the ratio of starch : gelatin has an impact on the solubility conditions, films with different ratios (4:1, 1:1 and 1:4) of starch : gelatin were prepared and analysed.

Two centimeter diameter samples were cut from the incorporated films. The initial dry weight of the samples was obtained by drying them at 105°C in a circulation oven. After the first weighing, the samples were immersed in a flask containing 50 ml of distilled water, and maintained under slow agitation for 24 h. The swollen samples were then removed and dried again at 105°C for 24 h before determining their final dry weight.

The solubility of the samples in acid medium was tested by the same procedure as done for water. After determining their initial dry weight; the

samples were immersed in a beaker containing hydrochloric acid (1M) for 24 h, and then dried and weighed to determine their final dry weight.

3.2 Preparation of the test organisms:

Staphylococcus aureus and *Klebsiella pneumoniae* were selected as test organisms in this experiment. A working culture was prepared by inoculating a loopful of the culture into 10 ml of Trypticase Soy broth. Two subcultures were prepared in the same way and a third sub culture was prepared in 50 ml Trypticase Soy broth. The working culture was prepared by pipetting 20 ml of this third 18hr-sub culture in 180 ml sterile normal saline solution, mixed thoroughly on a magnetic stirrer at 100 rpm for 10 minutes. These working cultures were used for infecting the *in vitro* models.

3.3 Preparation of the contaminated model food system:

Employing *in vitro* model systems would confirm the efficacy of any edible film to act against the microorganisms when applied in contaminated environments. Since several studies employing fruits and raw vegetables as model systems have provided the base for the successful development of antimicrobial edible films, the developed edible film from our study was tested for its efficacy in carrots.

Carrots were selected as model food systems for studying the antimicrobial activity of edible films incorporated with methanolic extract of the *Couroupita guianensis* flowers. The carrots were peeled off and cut into circle pieces of approximately 10g per piece, diameter approximately 2cm. Each piece was disinfected in 70% ethanol for 5 minutes and dried in a laminar air flow chamber for 10 minutes. Carrot samples were immersed in the working test organisms culture for 10 minutes by stirring, allowed to drip free of excess inoculum and dried for 20 minutes in a laminar air flow chamber.

These infected carrots were used as controls in the following procedures for comparing the inhibition potency of incorporated films.

3.4 Antimicrobial Edible Films on the Pre-contaminated Model Food systems

The antimicrobial films with various concentrations (50mg to 150 mg) of methanolic extracts of *Couroupita guianensis* were placed to both sides of the contaminated carrots. Each sample was placed in a sterile Petri dish. The Petri dishes were sealed with parafilm and separately stored at room temperature or at 4°C. The number of bacterial survivors was monitored after 0, 24 and 48 hours storage time. Contaminated samples without application of the antimicrobial films and samples with edible films without medicinal plant extract served as controls. After the storage times, the sample was homogenized with 90 ml of tryptone sodium chloride with neutralizer in a stomacher at the highest speed for 3 minutes. The solution was serially diluted and appropriate dilutions were surface plated on Trypticase soy agar. Plates were incubated at 37°C for 24 hours before enumeration.

4.0 RESULTS AND DISCUSSION

Extensive literature survey has exposed that *Couroupita guianensis* is an important medicinal plant with diverse ethno medical and pharmacological spectrum. Many valuable natural constituents occur in this plant which contributes to its wide ranging pharmacological properties.

A comprehensive evaluation needs to be done on *Couroupita guianensis* plant, as the various parts such as flowers, fruits have already been studied individually. This will help in finding out new formulations for specific practical applications. Use of polysaccharide biopolymers for formation of edible films which must be environmentally safe and non toxic have to be evaluated by standard analytical protocols.

The present study was done to develop a biofilm impregnated with extracts of the flowers of *Couroupita guianensis* to increase the shelf life of the vegetables. The results obtained from the study are discussed as follows.

4.1 Formation of Edible Film:

A successful biodegradable edible film was prepared (Plate 2) with the starch and gelatin solutions along with glycerol as the plasticizer. The glycerol, which when combined with polymers, reduces brittleness, impacts flow and flexibility and enhances toughness and strength of the films (Kafrani *et al.*, 2016). Thus a film which has a combination of polymers and plasticizer will reduce flaking and cracking by improving coating flexibility (Galus and Lenart, 2013)

Small size, high polarity, more polar groups per molecule and more distance between polar groups within a molecule are the characteristics of a plasticizer which enhance plasticizing effects on a polymeric system.

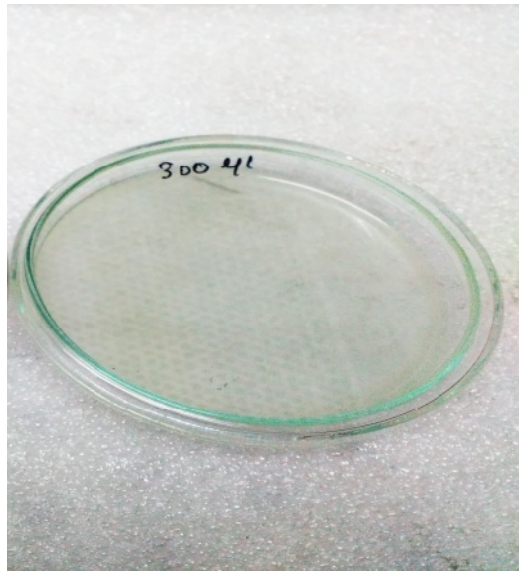


Plate 2: Developed Edible films

Due to the growing demand for biodegradable materials and for their production in greater volume, starch based films are preferred over other biopolymers. Starch based films exhibit excellent physical properties and has no smell, no taste and no color and impermeable to oxygen (Dhall, 2013). Moreover, starch imparts good tensile strength to the film.

It has been proved by Bomilla et al (2012) that starch polymers are excellent barriers to oxygen and carbon dioxide due to their tight packing and ordered hydrogen bonded network structure. This will eventually help in preventing dehydration, oxidative rancidity and surface browning (Dhanapal *et al.*, 2012).

Thus an edible film having favorable physicochemical and sensory properties was formed using a combination of starch gel and glycerol (which served as a plasticizer). Further, hydro gels are highly hydrophilic polymer gels with the ability of swelling by absorbing and retaining large amounts of water without dissolving or losing their integrity in water. Therefore, a combination of starch and gel along with plasticizer will be more effective for high moisture foods. Such coatings have been successfully used to extend storage life of strawberries (*Fragaria annanassa*) and also to maintain texture and surface color (Cazon *et al.*, 2016). Also, starch-gelatin based coats developed on Red crimson grapes have also been successful extending the shelf life with a maximum storage period of 21 days (Fakhouri *et al.*, 2015)

4.2 *Couroupita guianensis* incorporated edible films:

The methanolic extracts of the flowers of *Couroupita guianensis* was used to incorporate in the edible film. This is because of the pronounced antimicrobial activity exhibited by methanolic extracts when compared to the aqueous and chloroform extracts of the flowers, during previous studies.

In our experiment, 5 g of the flowers of *Couroupita guianensis* were taken for extraction, and the yield percentage obtained was found to be 1.2 g and it was dissolved in 2.5 ml of DMSO. These extracts were successfully

incorporated into the edible film and an antimicrobial edible film containing *Couroupita guianensis* extracts was developed which is shown in Plate 3.



Plate 3: *Couroupita guianensis* incorporated edible films

The incorporation of antimicrobial agents into the edible films and coatings has demonstrated to act as a stress factor to decrease pathogen growth and to protect food stuffs against spoilage flora by Kafrani *et al* (2016). There is a trend to select the antimicrobials from natural sources and use generally recognized as safe (GRAS) compounds so as to satisfy consumer demands for healthy foods, free of chemical additives (Melo *et al.*, 2013).

Since the antimicrobial activity of *Couroupita guianensis* extracts were already established, their effectiveness against the target microorganisms *Staphylococcus aureus* and *Klebsiella pneumoniae* will be confirmed when incorporated in edible film. Varying concentrations of the extract (50 - 150mg) were used during incorporation in the edible films to arrive at a final or highest concentration of the extract required to inhibit bacterial growth in subsequent procedures.

There are several instances in literature mentioning the development of edible films incorporated with plant extracts which have antimicrobial potency. A good example is the development of elaborate gelatin based edible films, with or without chitosan which incorporated oregano and rosemary essential oil. These films were tested for their preservative effect when applied on cold smoked sardine. Moreover, these films enriched with essential oils were able to slow lipid oxidation (Atares and Chiralt 2016).

Diverse studies with *Quillaja saponaria* extracts as antimicrobial agents in edible coatings have also lead to improved food safety and enhanced shelf life in strawberries. The antimicrobial activity of this plant extract was found to be exclusively due to its high phenolic content (Zuniga *et al.*, 2012).

Likewise, incorporating extracts of Mexican lime and oregano by Nydia *et al* (2013), have been found to reduce the growth of *Salmonella*, *Shigella* and *E. coli* organisms, which are frequently associated with outbreaks from contaminated leafy green vegetables.

4.2.1 Water and acid solubility analysis of edible films

Water and acid solubility of the developed edible film incorporated with the flower extracts of *C. guainensis* was done to analyse the solubility of the developed film when consumed. The percentage of water solubility obtained for the different ratios of starch and gelatin in the incorporated film are tabulated below.

Table 1: Water solubility of incorporated edible films

S.No	Ratio of starch: gelatin	Water solubility %
1	4:1	11.4±0.79
2	1:1	31.4±0.14
3	1:4	28.1±1.66

Analyzing the data from the above Table 1, it could be said that when more gelatin is present in the formulation, greater amounts of water may be absorbed by the films, considering its hydrophilic nature. This in turn relates to increased film solubility. The developed edible film incorporated with the flower extracts of *Couroupita guianensis* thus had a high water solubility percentage of 31.4 with increase in gelatin content. Therefore, an optimum 1: 1 ratio of starch: gelatin was chosen as the best ratio for the development of *Couroupita guianensis* incorporated film.

The results of acid solubility analysis revealed that the flower extracts of *Couroupita guianensis* incorporated edible films were completely soluble in acid medium. This is an important fact to be considered, because, this acidity simulates the conditions of our mouth, suggesting that the coating would be completely dissolved when consumed (Fadini, 2013).

Similar results have been shown by Fakhouri *et al* (2012), where combinations of manioc starch and gelatin had higher solubility when gelatin content was raised. This was successfully developed and applied for Red Crimson grapes (Fakhouri *et al.*, 2015).

Thus the prepared edible film incorporated with *Couroupita guianensis* extract proved to have a significantly high water and acid solubility when an equal ratio of starch: gelatin was used, which is very advantageous for commercial applications.

4.2.2 *Invitro* model systems with carrot:

Carrots were used as models to determine the inhibition potency of the extracts of *Couroupita guianensis* against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Placement of the developed edible films on either side of the pre- contaminated carrots was done and the inhibition potency of film

was evaluated at 0th, 24th and 48th hour respectively in petriplates containing different concentration of the extract (50-150mg).(Plate 4)

Homogenates of carrots with these varying concentrations of extracts were evaluated for reductions in microbial populations. The results obtained by plating the serial dilutions of the homogenates are presented as follows for 0th, 24th and 48th for each organism respectively.

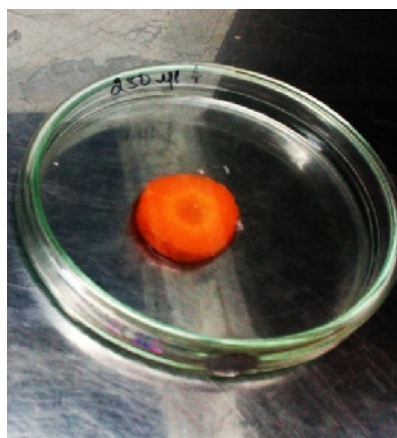


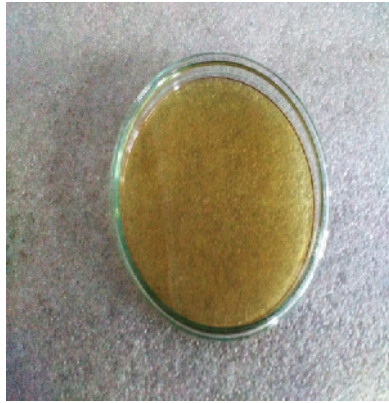
Plate 4: Edible film Coated Carrots as in vitro model system

Table 2: Preservative efficiency of developed edible films at 0th hour in *Staphylococcus aureus* infected carrots

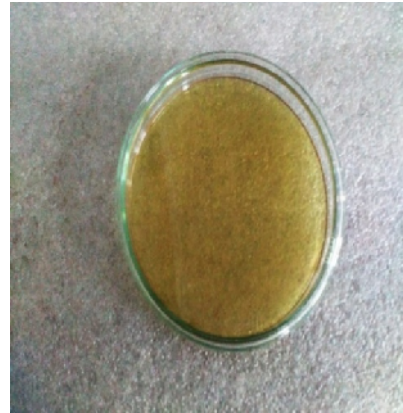
S.No	Concentration of plant extract in developed edible film(mg)	CFU for serial dilutions of test homogenate					
		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
1.	Control	TNTC	290	240	220	150	147
2.	50	117	103	97	86	80	70
3.	75	88	75	70	68	50	53
4.	100	80	66	57	48	46	30
5.	125	50	37	34	28	20	16
6.	150	28	22	20	11	5	2

The results from the Table 2 and Plate 5 show that the antibacterial potential of *Couroupita guianensis* flower incorporated edible film was effective when compared to the control group. Among the various dilutions, the number of colony forming units was found to gradually decrease as the dilutions increased to 10⁻⁶.

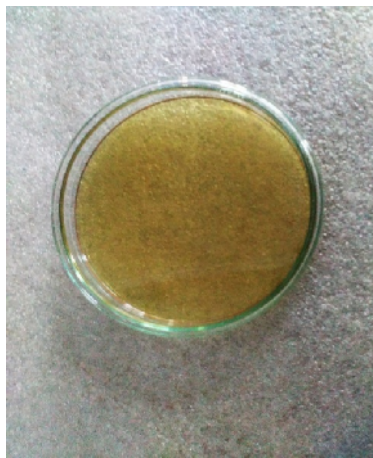
The different concentrations of the flower extract incorporated in the edible film showed various inhibitory levels, but at the maximum concentration of 150mg, the number of colonies had reduced to 2, for the dilution of 10⁻⁶; whereas there was 147 CFU for the same dilution in the control. It can be said that the number of CFUs of *Staphylococcus aureus* has been drastically controlled by the *Couroupita guianensis* flower extract incorporated edible film in the pre contaminated carrot model.



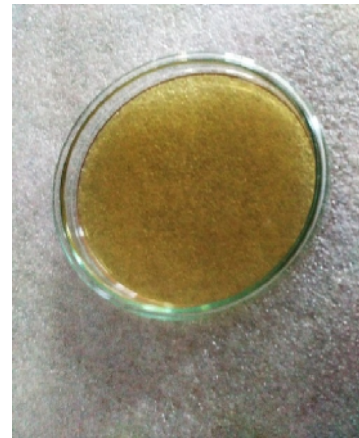
10⁻¹ dilution



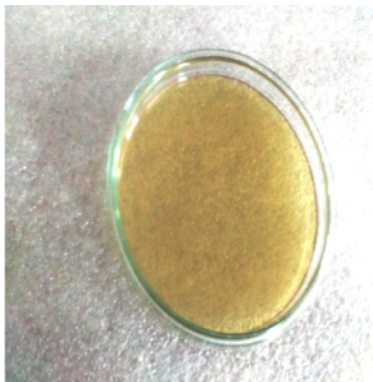
10⁻² dilution



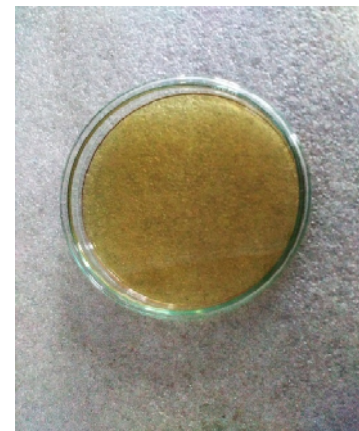
10⁻³ dilution



10⁻⁴ dilution



10⁻⁵ dilution



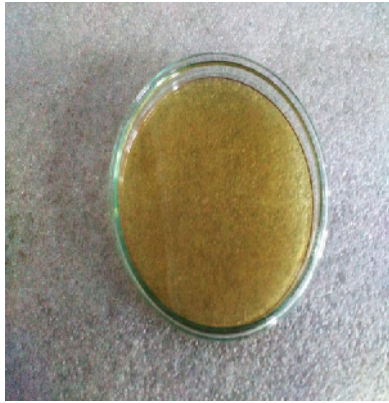
10⁻⁶ dilution

Plate 5: Inhibitory effects of edible film *Staphylococcus aureus* at 0th hour

Table 3: Preservative efficiency of developed edible films at 24th hour in *Staphylococcus aureus* infected carrots

S.No	Concentration of plant extract in developed edible film(mg)	CFU for serial dilutions of test homogenate					
		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
1.	Control	TNTC	TNTC	TNTC	270	200	180
2.	50	230	208	200	153	128	106
3.	75	105	97	90	85	82	76
4.	100	90	86	76	70	63	58
5.	125	60	52	41	46	30	24
6.	150	33	32	26	17	8	4

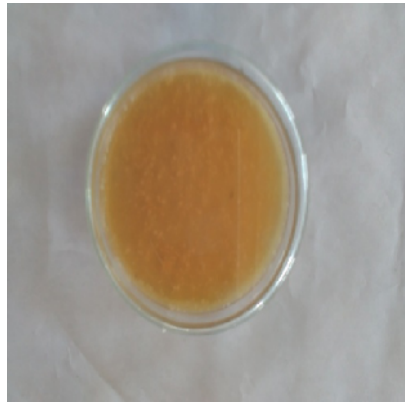
Table 3 and Plate 6 shows that the inhibition potency of *Couroupita guianensis* incorporated edible film was effective at 24th hour when compared with the controls. The CFUs decreased gradually as the dilutions reduced indicating that the inhibition was prominent. The number of colonies was found to be 4 when compared to control, which was about 180 CFUs at 10⁻⁶ dilution.



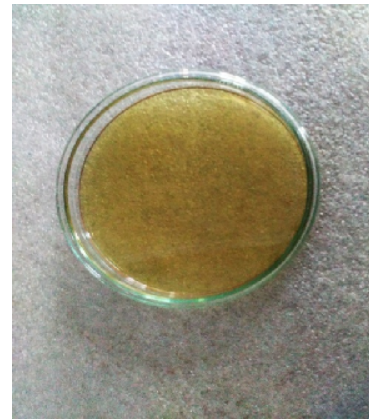
10^{-1} dilution



10^{-2} dilution



10^{-3} dilution



10^{-4} dilution



10^{-5} dilution



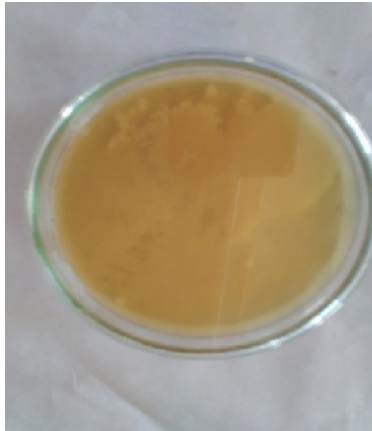
10^{-6} dilution

Plate 6: Inhibitory effects of edible film on *Staphylococcus aureus* at 24th hour

Table 4: Preservative efficiency of developed edible films at 48th hour in *Staphylococcus aureus* infected carrots

S.No	Concentration of plant extract in developed edible film(mg)	CFU for serial dilutions of test homogenate					
		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
1.	Control	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
2.	50	275	143	129	98	62	58
3.	75	90	86	80	63	51	26
4.	100	75	72	64	56	42	22
5.	125	72	59	55	50	26	8
6.	150	60	57	52	32	10	5

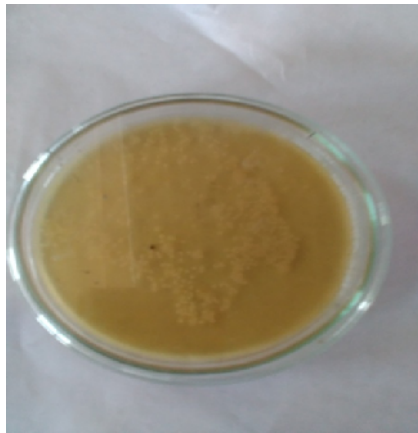
Table 4 and Plate 7 shows that the inhibition potency of *Couroupita guianensis* incorporated edible film was effective at 48th hour too when compared with the controls. The CFUs decreased gradually as the dilutions reduced indicating that the inhibition was prominent. The number of colonies obtained for a concentration of 150 mg was 5 when compared to control, which was about TNTC CFUs at 10⁻⁶ dilution.



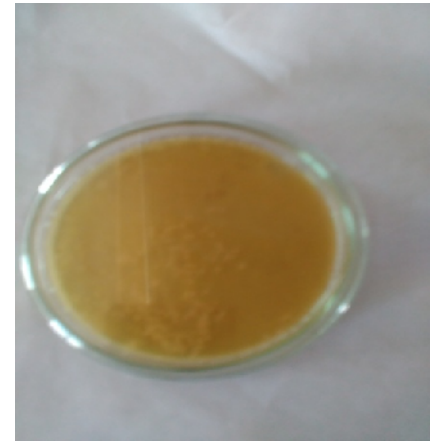
10⁻¹ dilution



10⁻² dilution



10⁻³ dilution



10⁻⁴ dilution



10⁻⁵ dilution



10⁻⁶ dilution

Plate 7: Inhibitory effects of edible film on *Staphylococcus aureus* at 48th hour

Table 5: Preservative efficiency of developed edible films at 0th hour in *Klebsiella pneumoniae* infected carrots

S.No	Concentration of plant extract in developed edible film(mg)	CFU for serial dilutions of test homogenate					
		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
1.	Control	TNTC	TNTC	246	220	167	120
2.	50	123	110	98	74	70	63
3.	75	86	70	70	65	63	50
4.	100	45	42	36	34	30	26
5.	125	30	23	21	17	11	8
6.	150	17	14	11	8	3	2

From the Table 5 and Plate 8 it was found that the inhibition potency of *Couroupita guianensis* incorporated edible film was effective at 0th hour when compared with the controls. This was similar to the inhibition trend obtained for *S. aureus* at 0th hour. The CFUs decreased gradually as the dilutions reduced indicating that the inhibition was significant for *Klebsiella pneumoniae*. The number of colonies obtained for a concentration of 150 mg was only 2 when compared to control, which was about 120 CFUs at 10⁻⁶ dilution.



10⁻¹ dilution



10⁻² dilution



10⁻³ dilution



10⁻⁴ dilution



10⁻⁵ dilution



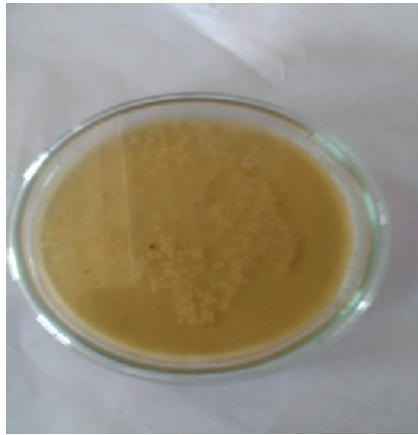
10⁻⁶ dilution

Plate 8: Inhibitory effects of edible film on *Klebsiella pneumoniae* 0th hour

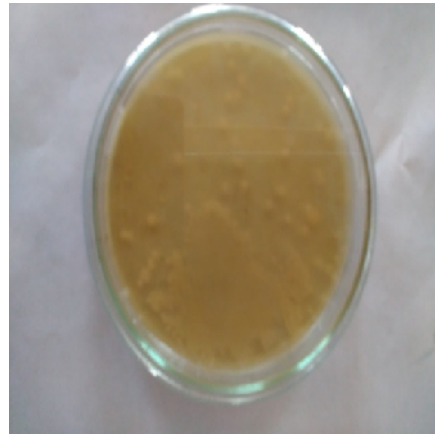
Table 6: Preservative efficiency of developed edible films at 24th hour in *Klebsiella pneumoniae* infected carrots

S.No	Concentration of plant extract in developed edible film(mg)	CFU for serial dilutions of test homogenate					
		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
1.	Control	TNTC	TNTC	TNTC	267	260	200
2.	50	220	210	186	157	143	103
3.	75	107	100	92	88	86	73
4.	100	90	85	80	71	46	40
5.	125	59	48	40	35	23	20
6.	150	38	29	23	17	11	3

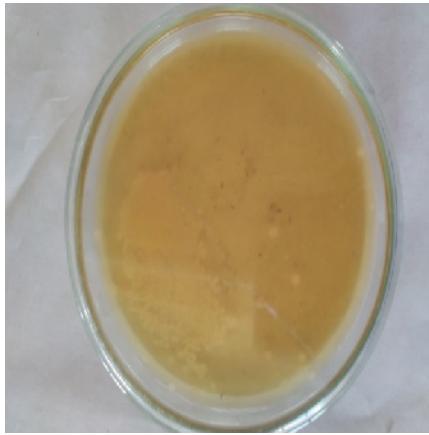
The Table 6 and Plate 9 show that the inhibition potency of *Couroupita guianensis* incorporated edible film was effective at 24th hour when compared with the controls. The CFUs decreased gradually as the dilutions reduced following the same trend of *S. aureus*, indicating that the inhibition was significant for *Klebsiella pneumoniae*. The number of colonies obtained for a concentration of 150 mg was 3 when compared to control, which was about 200 CFUs at 10⁻⁶ dilution.



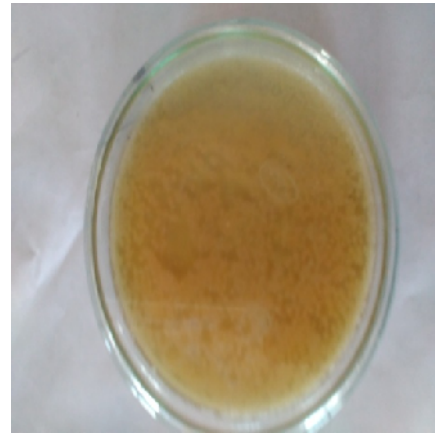
10^{-1} dilution



10^{-2} dilution



10^{-3} dilution



10^{-4} dilution



10^{-5} dilution



10^{-6} dilution

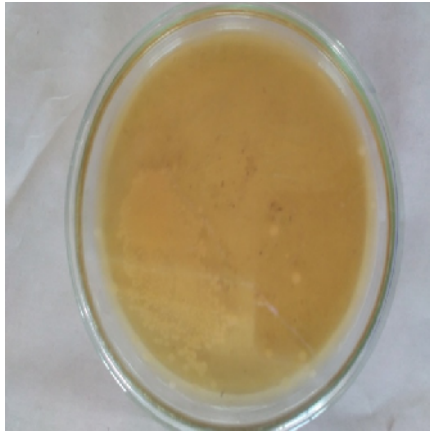
Plate 9: Inhibitory effects of edible film on *Klebsiella pneumoniae* at 24th hour

Table 7: Preservative efficiency of developed edible films at 48th hour in *Klebsiella pneumoniae* infected carrots

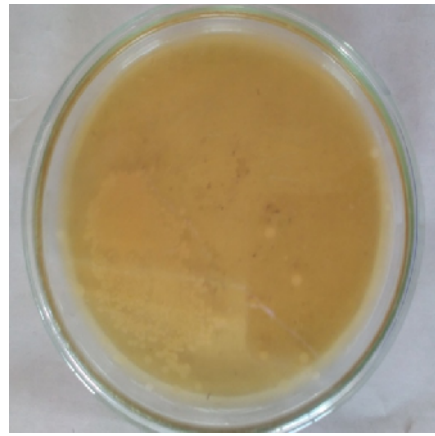
S.No	Concentration of plant extract in developed edible film(mg)	CFU for serial dilutions of test homogenate					
		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
1.	Control	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
2.	50	280	256	211	187	168	150
3.	75	127	120	107	89	70	63
4.	100	103	97	90	85	67	33
5.	125	65	57	45	40	27	22
6.	150	58	36	20	17	12	5

From the Table 7 and Plate 10 shows that the inhibition potency of *C. guainensis* incorporated edible film was effective at 48th hour when compared with the controls. The CFUs decreased gradually as the dilutions reduced indicating that the inhibition was significant for *K. pneumoniae*. The number of colonies obtained for a concentration of 150 mg was 5 when compared to control, which was TNTC CFUs at 10⁻⁶ dilution.

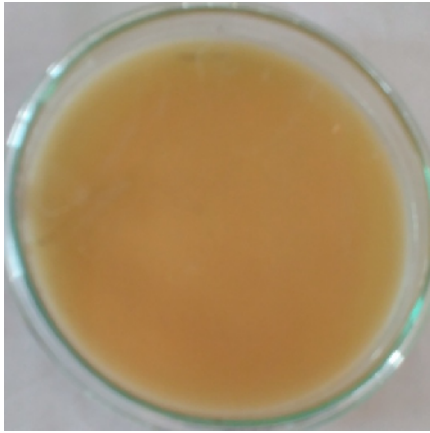
A compound is recognized as having a strong bactericidal effect when it is capable of causing a 1000 fold reduction (3 orders of magnitude or 99.9%) of the initial inoculums (Melo *et al.*, 2013). From the tables 5 and 8, it could be seen that there was considerable reduction of *Staphylococcus aureus* and *Klebsiella pneumoniae* in the microbial population size at 48th hour. This represents a 3-4 fold magnitude reduction in the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae* when compared with control sample which has TNTC CFU/ml.



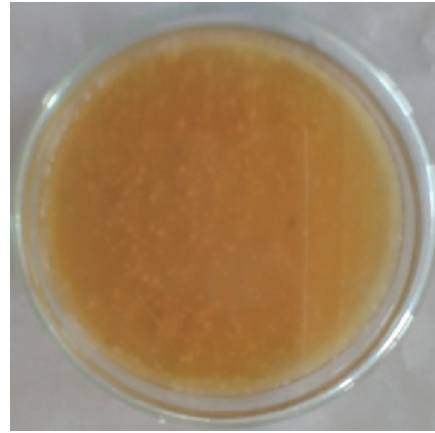
10⁻¹ dilution



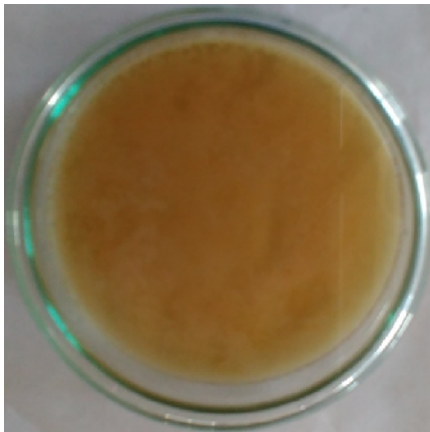
10⁻² dilution



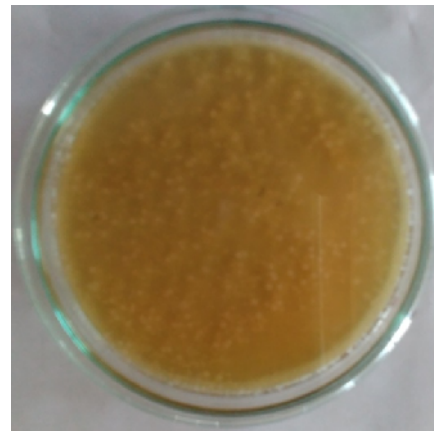
10⁻³ dilution



10⁻⁴ dilution



10⁻⁵ dilution



10⁻⁶ dilution

Plate10: Inhibitory effect of edible films on *Klebsiella pneumoniae* at 24th hour

150mg of the extract incorporated biofilm was more effective in inhibiting the growth indicating the highest concentration of the extract which is required to achieve antimicrobial effectiveness in *invitro* model.

Similar results were obtained in *invitro* models using meat, where the essential oils from *Couroupita blanchetianus* were tested for antimicrobial efficacy (Melo *et al.*, 2013)

The same 3-4 fold magnitude reduction was also witnessed for the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae* when compared with controls. Thus, *Staphylococcus aureus* and *Klebsiella pneumonia* were inhibited efficiently by *Couroupitaguainensis* flower extracts.

Dhabi *et al.*, (2012) showed antimicrobial, antimycobacterial and antibiofilm properties in chloroform extract of the fruit of *Couroupitaguainensis*. Moreover an effective antibiofilm activity by the chloroform extracts against gram negative microorganism *Pseudomonas aeruginosa* was also observed. The antimicrobial property of the methanolic extracts of *Couroupitaguainensis* flowers was also conjointly showed by Ramalakshmiet *et al.*, 2013

An effective repressing activity against *Plesiomonas shigelloides*, *Vibrio mimicus* and *Proteus vulgaris* has been recorded and a moderate antimicrobial activity for *E.coli*, *Klebsiella pneumoniae* and *Salmonella typhi* was found (Regina and Rajan *et al.*, 2012).

Methanol extracts of Turkish plants, *Ficus carica*, *Juglans regia*, *Olea eurpeae*, *Punica granatum* and *Rhus ciliaria* have been found to effectively inhibit food related bacteria such as *L. monocytogenes* and *S. aureus*. This was attributed to the high polyphenol content and good quercitrin, myricetrin content. (Nostro *et al.*, 2016). Therefore, *in vitro* activity of methanolic plant extracts provide insights about the antibacterial, antibiofilm and antioxidant activities.

Plant antimicrobial compounds such as carvacrol and cinnamaldehyde have been found to act efficiently against *Salmonella* when incorporated in edible films. These were developed and successfully used in apples.

The inhibitory activities against the microbes are due to the unique phytoconstituents present in *Couroupita guianensis*, some of which have been discovered earlier. Few chemical studies on this species has revealed the presence of α -amirin, β -amirin, β -sitosterol, tannins (Sridar, S., *et al.*, 2011); (Bergman *et al.*, 1985), Ketosteroids and terpenoids and alkaloids, carbohydrates and proteins (Ramalakshmi *et al.*, 2013). In the *Couroupita guianensis* flowers, compounds such as Eugenol, volatile oil and (E,E)-Farnesol were categorized. Compounds such as indigo, indirubin, linoleic acid, nerol, Tryptanthrin were also found to be exclusively present in the flowers and fruits of *Couroupita guianensis* (Bergman *et al.*, 1985)

Eugenol has been found to combat *E.coli* effectively when used as natural food antimicrobials for prolonging shelf life of apple juices (Azimi *et al.*, 2012). Since the flower extracts of *Couroupita guianensis* contain Eugenol, the antimicrobial potential exhibited against *Staphylococcus aureus* and *Klebsiella pneumonia* may be attributed to this fact.

This proves that the natural extracts of the flowers of *Couroupita guianensis* when incorporated in a stable, biodegradable edible film can be promising preservatives for use in food products which can efficiently preserve vegetables and extend their shelf life.

5.0 SUMMARY AND CONCLUSION

Edible coatings are an environmentally friendly technology that is applied on many products to control moisture transfer, gas exchange or oxidation processes. Edible coatings can provide an additional protective coating to produce and can also give the same effect as modified atmosphere storage in modifying internal gas composition. One major advantage of using edible films and coatings is that several active ingredients can be incorporated into the polymer matrix and consumed with the food, thus enhancing safety or even nutritional and sensory attributes.

Plant derived essential oils and extracts have long been used as a food additive to prolong food shelf life by preventing rancidity and controlling microbial contamination, due to their high content of secondary metabolites mainly Phenolic compounds such as isoflavanoids, terpenes, ketones, aliphatic alcohols, acids, aldehydes.

Earlier studies on antimicrobial potential of the leaves, flowers, fruit and bark of the plant *Couroupita guianensis* have been carried out in our laboratory. Among the various bacteria tested for their susceptibility to the methanolic extract of *Couroupita guianensis*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were found to be most susceptible. Hence, the present study focused on formation of a good biodegradable edible film incorporated with *Couroupita guianensis* flower extract which can efficiently inhibit *Klebsiella pneumoniae* and *Staphylococcus aureus*. The efficacy of the developed edible film was confirmed using carrot as *in vitro* model system.

Use of polysaccharide biopolymers was employed for the formation of edible films which must be environmentally safe and non toxic. Thus, by using starch and gel solutions, a successful biodegradable edible film was prepared along with glycerol as the plasticizer. Since a film which has a combination of polymers and plasticizer will reduce flaking and cracking by improving coating flexibility, the developed edible film was found to be a stable biopolymer.

The methanolic extracts of the flowers of *Couroupitaguainensis* was used to incorporate in the edible film. The yield obtained from 5 g of the flowers of *Couroupita guianensis* during methanol extraction, was found to be 1.2 g and it was dissolved in 2.5 ml of DMSO. These extracts were successfully incorporated into the edible film and an antimicrobial edible film containing *Couroupita guianensis* extracts was developed.

Water and acid solubility of the developed edible film incorporated with the flower extracts of *C. guianensis* was done to analyse the solubility of the developed film when consumed. The developed edible film incorporated with the flower extracts of *Couroupita guianensis* thus had a high water solubility percentage of 31.4 with increase in gelatin content. Therefore, an optimum 1: 1 ratio of starch: gelatin was chosen as the best ratio for the development of *Couroupita guianensis* incorporated film.

Further, acid solubility analysis revealed that the flower extracts of *Couroupita guianensis* incorporated edible films were completely soluble in acid medium. Acidity simulates the conditions of our mouth, suggesting that the coating would be completely dissolved when consumed.

Carrots were used as models to determine the inhibition potency of the extracts of *Couroupita guianensis* against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The developed edible films were placed on either side of the pre- contaminated carrots and the inhibition potency of film was evaluated at 0th, 24th and 48th hour respectively in petriplates containing different concentration of the extract (50-150mg).

Homogenates of carrots with these varying concentrations of extracts were evaluated for reductions in microbial populations. Plating the serial dilutions of the homogenates for 0th, 24th and 48th for each organism revealed the inhibition potency of the incorporated edible film.

The different concentrations of the flower extract incorporated in the edible film showed various inhibitory levels, but at the maximum concentration

of 150mg, the number of colonies reduced to 2, for the dilution of 10^{-6} , whereas there was 147 CFU for the same dilution in the control. It can be said that the number of CFUs of *S. aureus* has been drastically controlled by the *Couroupita guianensis* flower extract incorporated edible film in the pre contaminated carrot model.

The same trend was seen in the 24th and 48th hour homogenates, where 2 and 5 CFUs were obtained respectively. This was much less when compared to the controls which had TNTC colonies. For the tests with *K. pneumoniae*, the CFUs decreased gradually as the dilutions reduced following the same trend of *S. aureus*, indicating that the inhibition was also significant for *Klebsiella pneumoniae*. This represents a 3-4 fold magnitude reduction in the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae* when compared with control sample which has TNTC CFU/ml.

Thus, it was found that 150mg of the extract incorporated biofilm was more effective in inhibiting the growth indicating the highest concentration of the extract which is required to achieve antimicrobial effectiveness in *in vitro* model. The inhibitory activities against the microbes are due to the unique phytoconstituents present in *Couroupita guianensis*, some of which have been discovered earlier.

Since the flower extracts of *Couroupita guianensis* contain Eugenol, farnesol and other volatile oils, the antimicrobial potential exhibited against *Staphylococcus aureus* and *Klebsiella pneumoniae* may be attributed to this fact.

Therefore, it can be concluded that the developed edible film incorporated with *Couroupita guianensis* extracts serves to be an efficient film capable of protecting and elongating the shelf life of vegetables against pathogenic microorganisms. Further studies can be carried out to confirm its potential against various bacteria and employing different *in vitro* model.

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