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PREPARATION AND CHARACTERIZATION OF PECTIN AEROGELS LOADED WITH *Senna alata (L.) Roxb.* LEAVES EXTRACT

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DECLARATION

I hereby declare that this thesis entitled "Preparation and Characterization of Pectin Aerogels Loaded with *Senna alata (L.) roxb*. Leaves Extract" is the result of my research except as cited in references.

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PENYEDIAAN DAN PENCIRIAN AEROGELS PECTIN YANG DIISI DENGAN EKSTRAK DAUN *SENNA ALATA (L.) Roxb.*

ABSTRAK

Dalam kajian ini, pendekatan untuk menyediakan, menghasilkan dan mencirikan aerogel pektin yang dimuatkan dengan ekstrak daun *Senna alata (L.) roxb* telah dijalankan. Tujuan penyelidikan ini adalah untuk mengekstrak daun senna alata dan menggabungkan pengekstrakan dengan pektin aerogel untuk diaplikasikan dalam industri pembungkusan makanan. Kajian tertumpu kepada penyediaan dan pencirian aerogel pektin yang dimuatkan dengan Senna Alata (L.) roxb. ekstrak daun. Kajian ini bertujuan untuk menyiasat kesan Senna Alata (L.) roxb. ekstrak pada sifat fizikal, kimia, haba dan antimikrobial pektin aerogel sifat antimikrobial 5wt% dan 10wt% aerogel pektin dicampurkan dengan 0%, 0.5%, 1.0%, 1.5%, dan 2.0% daripada Senna Alata (L.) perahan daun roxb yang telah digabungkan dengan aerogel. Kepentingan kajian ini terletak pada potensi aerogel pektin sebagai alternatif yang mampan dan berfaedah kepada alam sekitar untuk kusyen penyerap pembungkusan makanan. Hasil pencirian menunjukkan kedua-dua 5wt% dan 10wt% aerogel pektin yang digabungkan dengan ekstrak daun *Senna alata (L.) roxb* yang ditunjukkan adalah hasil yang sangat baik. Oleh itu, daripada semua pencirian aerogel pektin dengan perahan daun Senna Alata (L.) roxb, hasil 10wt% menunjukkan hasil terbaik untuk digunakan dalam pembungkusan makanan.

Kata Kunci: Pektin aerogel, *Senna alata (L.) roxb*, antimikrobial, TGA, FTIR

PREPARATION AND CHARACTERIZATION OF PECTIN AEROGELS LOADED WITH *Senna alata (L.) roxb*. LEAVES EXTRACT

ABSTRACT

In this study, an approach to prepare, produce, and characterize pectin aerogels that were loaded with *Senna alata (L.) roxb* leaves extraction was conducted. The purpose of this research was to extract the leaves of senna alata and incorporated the extraction with pectin aerogels for application in food packaging industries. The study focused on the preparation and characterization of pectin aerogels loaded with *Senna Alata (L.) roxb*. leaves extract. The study aims to investigate the effect of *Senna Alata (L.) roxb*. extract on the physical, chemical, thermal, and antimicrobial properties of pectin aerogel antimicrobial properties of 5wt% and 10wt% of pectin aerogels loaded with 0%, 0.5%, 1.0%, 1.5%, and 2.0% of *Senna alata (L.) roxb* leaves extraction that was incorporated with into aerogels. The significance of the study lies in the potential of pectin aerogels as a sustainable and environmentally beneficial alternative for food packaging absorbent cushioning. The result of the characterization indicated both 5wt% and 10wt% of pectin aerogels that incorporated with *Senna alata (L.) roxb* leaves extraction shown are very good results. Hence, from all the characterization of pectin aerogels with *Senna alata (L.) roxb* leaves extraction, the result of 10wt% showed the best result to use in food packaging.

Keywords: Pectin aerogel, *Senna alata (L.) roxb*, antimicrobial, TGA, FTIR

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LIST OF ABBREVIATIONS

DPPH	α-diphenyl-β-picrylhydrazyl
Ph	potential of hydrogen
BPA	bisphenol A
BHA	butylated hydroxy anisole
BHT	butylated hydroxytoluene
PG	propyl gallate
TBHQ	tert-butyl hydroquinone
RG-I	rhamnogalacturonan I
HG	homogalacturonan
RG-II	rhamnogalacturonan II
WT%	percentage by weight
RCA	root cause analysis
TiO2	titanium oxide
kJ/kgK	Kilojoule per kilogram kelvin
ROS	Reactive Oxygen Species
DNA	Deoxyribonucleic Acid
°C	degree Celsius
ML	millimetre

RPM	Revolutions per minute
HCl	hydrochloric acid.
KOH	Potassium hydroxide
NaCl	sodium chloride
Na+	sodium ion
Ca2+	calcium cation
Mol	Molar
CO2	carbon dioxide
SEM	Scanning Electron Microscopy
FEG	Field Emission Gun
KeV	kilo-electron-volt.
FTIR	Fourier transform infrared spectroscopy.
TGA	thermogravimetric

LIST OF SYMBOLS

%	PERCENTAGE
°	DEGREE
cm	CENTIMETER
m	METER
mℓ	MILLIMETER
cm^2	CENTIMETER SQUARE
cm^3	CENTIMETER CUBIC
m^2	METER SQUARE
m^3	METER CUBIC
kJ/kgK	KILOJOULE PER KILOGRAM KELVIN

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Aerogels are highly porous and lightweight nanostructured materials composed of a solid network with air in the pores. Aerogels are usually obtained from a gel in which the liquid phase is replaced by air. To prevent the collapse of network structure during drying due to capillary forces, drying in supercritical (SC) conditions is performed. Classical aerogels based on silica, synthetic polymers and their carbons typically possess low density (< 0.2 g/cm^3), open pores in the range of small macro- and mesopores and very high specific surface area (700 - 1500 m^2/g) (Groult & Budtova, 2018). These structural properties make aerogels attractive materials for a broad variety of applications such as thermal and acoustic insulation, catalyst support, fuel cells, capacitors, absorption and adsorption, flame retardancy, ultrasound probes and ion exchange media (Groult & Budtova, 2018).

Bio-aerogels are new versatile materials based on polysaccharides. The latter are widely available, renewable, non-toxic, biocompatible and can be easily functionalized due to a large amount of hydroxyl groups on polymer backbone. Bio-aerogels are thus suitable for a wide range of life science applications such as biomedical, pharmaceutical, biotechnological, cosmetic and food (Manzocco et al., 2021). There are three different morphological forms of aerogel: monolith, powder, and film. Depending on the method of manufacture, the product is referred to as aerogel, xerogel, cryogel, or hydrogel. Aerogel is classified according to its microstructure as either microporous (with pores no larger than 2 nm) or mesoporous (with pores between 2 and 50 nm) or mixed porous.

Pectin aerogel is a type of aerogel, which is a low-density, very porous material that is made from gel. Pectin aerogels also produced via the gelation method, in which pectin is combined with a solvent, such as water or ethanol, and a cross-linking agent, such as calcium ions or citric acid. The mixture is then typically desiccated by supercritical drying, which involves removing the solvent under high pressure and

temperature to create a porous solid material. Pectin aerogel also one of the bio-based aerogels. For example, bio-aerogels serve as carrier matrices for various compounds and have enormous potential for micronutrient enrichment of foods. So, there will have a cross-linking with some extraction to produce the natural aerogel from natural resources. To make the absorbent pads in food packaging having a quality of biodegradable properties if there have one kind of quality like high of antioxidants, and also have antimicrobial.

Senna alata (L.) roxb exhibit the antimicrobial properties of the extraction of the leaves with methanol or ethanol. Vitamin C, flavonoid compounds, DPPH radical scavenging activity, and significant antioxidant activity against hydrogen peroxide and superoxide anion are just some of the antioxidants found in high concentrations in methanol extracts of *Senna alata (L.) roxb* flower and leaf. *Senna alata (L.) roxb* leave extract that rich with antioxidant compounds made them a promising/suitable candidate as an active compound of porous in food packaging. The bark and crushed leaves of *Senna alata (L.) roxb* are used to treat skin diseases caused by the ringworm parasite, and the seeds are used as an anthelminthic (Ibrahim and Osman, 1995).

Aerogel's porous structure enables absorbent cushions to absorb any condensation that food in storage may produce. These pads typically consist of a non-permeable or non-stick synthetic polymer, such as polyethylene, and a hydrophilic non-woven bottom layer filled with active compounds that inhibit bacterial development, such as citric acid and sodium bicarbonate (McMillin, 2017). Pectin aerogels have several potential applications, including smart packaging materials and consumable delivery systems for nutraceuticals, dietary supplements, flavouring, and other additives. It is possible to produce aerogels capable of transporting or hosting consumable substances. They can increase the ingredient's stability, mask its odor, and initiate its release at a certain pH level or after a certain period (Manzocco et al., 2021).

1.2 PROBLEM STATEMENT

Currently, in traditional ways is known as conventionally, absorbent pads in food packaging used polymer based and petroleum based which is one other disadvantages of using plastic based in food packaging is poor longevity as plastic degrades over time. Plastic is affected over time by scratches, being dented or cracked. Other than that, is possibly higher risk for high levels of BPA and also large environmental and health impact and can leading causes of pollution. And obviously the plastic-based food packaging in pads absorbance could not degradable and not biocompatible.

Therefore, the other problem that occurs because of conventional food packaging is the use of synthetic antioxidants, which are not very good for the food in the packaging and have a health impact on someone who eats the food from the packaging. Examples of synthetic antioxidants are butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ) (Lourenço et al., 2019).

Since both pectin aerogels and *Senna Alata (L.) roxb.* extraction can contribute to absorbency, compatibility, and antioxidant potential, this combination appears promising for food packaging absorbent cushioning. Thus, it is possible to use bio-based aerogels as absorbent coverings in food packaging. Especially with the use of natural resources and the inclusion of antioxidant- and antibacterial-rich *Senna alata (L.) roxb* leaf extract.

1.3 OBJECTIVES

In this research, there two objectives which need to be achieved. The objectives are:

1) To prepare pectin aerogel loaded with *Senna alata (L.) roxb* extract.
2) To study the effect of *Senna alata (L.) roxb* extract on the physical, chemical, thermal, and antimicrobial properties of pectin aerogel.

1.4 SCOPE OF STUDY

The scope of the study to includes the preparation of pectin aerogel and the extraction of plant material from *Senna alata (L.) roxb* leaves by using Soxhlet extraction. The plant extract from *Senna alata (L.) roxb* is then incorporate into the pectin aerogel to create a sustainable and environmentally beneficial. Pectin aerogel incorporate loaded with different concentration of *Senna alata (L.) roxb* extraction and was characterized based on its physical, chemical, biological, and thermal properties. Thus, the effect of pectin aerogel with *Senna alata (L.) roxb* was studied and observed.

1.5 SIGNIFICANCE OF STUDY

The present study is significant in several ways. Firstly, pectin aerogel has been identified as a promising alternative to conventional for food packaging due to its biodegradability, biocompatibility, antimicrobial, and antioxidant properties. However, there is a lack of research on the preparation and characterization of pectin aerogels loaded with antioxidant including the extraction of *Senna alata (L.) roxb* leaves extraction, characterization, and method. Therefore, this study aims to preparation and characterization of pectin aerogels loaded with *Senna alata (L.) roxb* extraction.

CHAPTER 2

LITERATURE REVIEW

2.1 PECTIN

Pectin is known as heteropolysaccharide is a type of soluble fibre found in a variety of fruits and vegetables, particularly the peels and centres of apples, citrus fruits, and plums. It is a complex carbohydrate and a type of hydrocolloid that, when combine with sugar and acid in specific ways. The most essential physical characteristic of pectin is its ability to form gels that can be spread. When polymer chains interact along a portion of their length to form a three-dimensional network, gel formation occurs. Pectin is a polysaccharide composed of D-glucopyranosyl uronic acid units connected by -D (1->4) glycosidic bonds (BeMiller, 1986). The primary chemical component of pectin is galacturonic acid, a sugar acid. Pectin is an abundant complex polymer present in the primary cell membranes of all land plants. Pectin is the most abundant structural protein in the middle lamella, where it also serves as a cell adhesive. There three main pectic polysaccharides are rhamnogalacturonan I (RG-I), homogalacturonan (HG), and rhamnogalacturonan II (RG-II) (O'Neill et al., 2022).

2.2 PECTIN AEROGEL

Pectin aerogel is a type of aerogel, which is a low-density, very porous material that is made from gel. Pectin aerogels also produced via the gelation method, in which pectin is combine with a solvent, such as water or ethanol, and a cross-linking agent, such as calcium ions or citric acid. The mixture is then typically desiccated by supercritical drying, which involves removing the solvent under high pressure and temperature to create a porous solid material. The pectin aerogel with the antioxidant of *Senna alata (L.) roxb* extraction to make the aerogel for food packaging or food preserve. Changes in parameters such as pH, polymer content, non-solvent type, and concentrations of monovalent and polyvalent metal ion salts can alter the highly porous and nanostructure structure of pectin aerogels. Supercritical carbon dioxide was utilized in a dissolution-solvent-exchange-drying procedure to create highly porous and nanostructured pectin aerogels (Groult & Budtova, 2018).

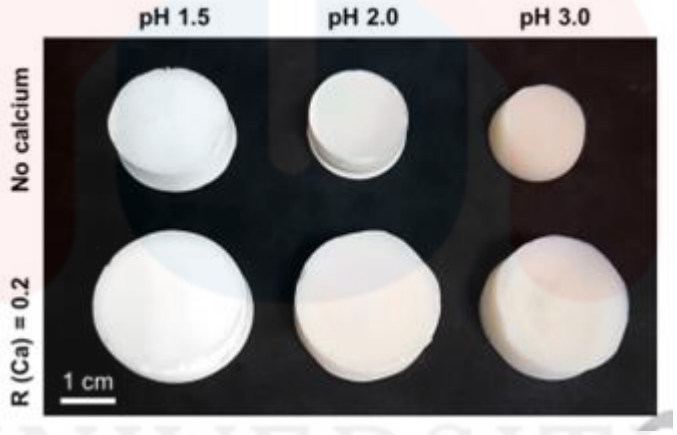

Figure 2.1: properties of pectin aerogels (Groult & Budtova, 2018)

Images of pectin aerogels fabricated from 3 wt.% solutions at pH 1.5, 2.0, and 3.0, without the addition of salts and with the addition of calcium at R(Ca) = 0.2. Before solvent exchange, the sample was a weak acid gel at pH 1.5, a high viscosity solution at pH 2.0, and a low viscosity solution at pH 3.0 in the absence of calcium. When R(Ca) = 0.2, strong ionic polymers were formed at all pH levels. Alcohol was not a solvent.

2.4 PROPERTIES OF PECTIN AEROGELS

2.3.1 PHYSICAL PROPERTIES

Pectin aerogels have unique physical properties that make them suitable for various applications such as thermal insulation and drug delivery, which is low density, typically the density of pectin aerogels ranges from 0.01 to 0.3 g/cm³ which is measure in gram per cubic centimetre. The following is high porosity, the pore volume in pectin aerogels approach 80% indicating of high porosity. These materials can utilise in applications such as adsorption, filtration, and catalysis due to their large surface area and low density (Groult & Budtova, 2018). And lastly, the lightweight of pectin aerogels are highly porous and lightweight nanostructured materials composed of a solid network with air in the pores.

2.3.2 CHEMICAL PROPERTIES

Aerogels are 99.8% porous, have low thermal conductivity, density, dielectric constant, and refractive index. Aerogels' startling properties enable many technological uses. Pectin was selected for the synthesis of pectin aerogels via dissolution-solvent-exchange-drying with supercritical carbon dioxide due to its tolerance to a broad spectrum of conditions that affect solution viscosity and gelation. Thus, because of the chemical properties of the pectin aerogels changes in external parameters (pH, polymer content, non-solvent type, and concentration of monovalent and polyvalent metal ion compounds) influenced the aerogel's shape, porosity, pore size, surface area, and mechanical properties (Groult & Budtova, 2018).

2.3.3 BIOLOGICAL PROPERTIES

Pectin aerogels have potential biological properties due to their turntable physical and mechanical properties. Due to their thermal resistance, mechanical properties, and antibacterial properties, pectin-based aerogels may be used in the food packaging industry. Mechanical and antibacterial testing were used to characterize pectin and pectin/TiO_2 nanocomposite aerogels, among other methods. The pectin aerogel used as the standard have a high thermal stability. Important biological characteristic of pectin aerogels is their biocompatibility. They are suitable for use in the food packaging industry, for example as an absorbency replacement, because they do not threaten living tissue. Pectin aerogels have been shown to be harmless to human cells, for instance. Additional research indicates that they promote cell proliferation and differentiation.

2.3.4 THERMAL PROPERTIES

Thermal conductivity is most important, followed by specific heat. Kistler sought accurate methods to study structural features including pore diameters. Scientists and engineers discovered early that aerogel is an excellent thermal insulation material. The thermal stability of pectin aerogels has outstanding thermal stability, typically withstanding temperatures between 200 and 300 degrees Celsius with minimal degradation. This characteristic is advantageous in situations where the material will be exposed to high temperatures, such as thermal sterilization procedures (Groult & Budtova, 2018). And lastly, the high specific heat capacity of pectin aerogels is another essential thermal property. Consequently, they are useful for applications such as thermal energy storage and heat barriers. The specific heat capacity of pectin aerogels is approximately 2.0 kJ/kgK, which is significantly greater than that of many other materials.

2.4 SENNA ALATA

The Fabaceae family includes the local favourite, *Senna Alata (L.) roxb*, better known as Gelenggang. Growing to a maximum height of 12 metres, this plant prefers the warm, humid climates of places like Africa and Southeast Asia. Skin infections, eczema, antiparasitic, bronchitis, asthma, and pain from dangerous insect bites are only some of the ailments that have been successfully treated with this plant (Lim et al., 2020). *Senna Alata (L.) roxb*, a plant with cathartic and antifungal properties, is included on the Thai government's list of approved plants for primary care. Twelve fresh or dried leaves are finely minced and boiled in two glasses of water until one glass of decoction is produced, which is then filtered and used as a laxative. When necessary, take the entire quantity of the concoction at once. Another method is to steep 1 to 2 teabags containing 3 grams of desiccated, powdered leaves in 2 to 5 minutes of boiling water before bedtime (Gritsanapan & Mangmeesri, 2009). Most of the majority the using of *Senna alata (L.) roxb* have been using it as traditional medicine in southeast Asian.

Figure 2.2: *Senna alata (L.) roxb* (Thierry Hennebelle et al., 2009)

2.5 BIOLOGICAL PROPERTIES OF SENNA ALATA

2.5.1 ANTIOXIDANTS ACTIVITY

Natural antioxidants have an important role in the prevention of many age-related diseases and promotion of health. Among natural antioxidants from plants, flavonoids and other phenolic compounds are potent antioxidants and chelating agents (Dehshahri et al., 2012). Antioxidants can help you from getting diseases that get worse over time, like cancer, heart disease, and Alzheimer's. ROS probably cause cell ageing, mutation, cancer, and coronary heart disease by making membranes less stable, breaking down DNA and proteins, and oxidising low-density lipoprotein (LDL). Antioxidants stop cell damage by stopping free radicals from being made. It has been demonstrated that *Senna alata (L.) roxb* extract with methanol possesses significant antioxidant activity, including Vitamin C, flavonoid compounds, DPPH radical scavenging activity, and potent antioxidant activity against hydrogen peroxide and superoxide anion.

2.5.2 ANTIMICROBIAL ACTIVITY

The antimicrobial activity of plants is primarily due to secondary metabolites. Antimicrobial phytochemicals comprise phenolics and polyphenols (flavonoids, quinones, tannins, coumarins), terpenoids, alkaloids, lectins, and polypeptides, among others. Especially in *Senna alata (L.) roxb* extraction, it has antimicrobial in the extraction which the function of antimicrobial as it has been demonstrated that *Senna alata (L.) roxb* has antifungal, antibacterial, laxative, hypoglycaemic, and diuretic properties. Antifungal properties of this plant are highly valued in the medical community.

2.6 APPLICATION OF PECTIN AEROGELS IN FOOD PACKAGING

While most aerogels are composed of inorganic or synthetic polymers like silica or metal oxides, any biopolymer can be converted into an aerogel. The second iteration of aerogels, specifically biopolymer-based aerogels with food-grade polysaccharides and proteins, facilitates the entry of these materials into the food industry (El-Naggar et al., 2020). Due to their unique physical properties, low toxicity, and compatibility with the human diet, these new pectin aerogel sources open an intriguing array of culinary applications. Aerogels have numerous potentials uses in the food industry, including intelligent food packaging components and consumable delivery systems for nutraceuticals, dietary supplements, flavours, and other additives. Using a pectin aerogel as a host or carrier can increase the chemical's stability, conceal any unpleasant odours, and provide a timed or pH-triggered release following ingestion (Betz et al., 2012).

CHAPTER 3

MATERIALS AND METHOD

3.1 MATERIALS

The materials that were used to prepared pectin aerogel were *Senna alata (L.) roxb* pectin powder, calcium chloride, distilled water, sieves, *Senna alata (L.) roxb* leaves extraction, Soxhlet apparatus, Solvent ethanol apparatus, ethanol, beaker, hot stir plate, blender, scale, measuring cylinder, and spoon. Since this research based by using pectin powder with the extraction of *Senna alata (L.) roxb* leaves, there will be four (4) different amounts of concentration of extraction and two (2) weight percentages (%). The apparatus and material will be providing.

3.2 METHODS

3.2.1 PREPARATION OF SENNA ALATA EXTRACTION

Fresh and healthy leaf of *Senna alata (L.) roxb* was collected from Lata Keding, Jeli, Kelantan, Malaysia. The plants were washed by used distilled water thoroughly to remove the foreign particles followed by dried the leaves using oven within 24 hours at 40-45°C. After that, the dried leaves were blend until turn into fine powder before being kept in airtight containers.

Taken the powder and pour it into the sieve to make sure the fine powder was separated from the unblended item. By using the first method which is Soxhlet extraction, set up the apparatus and the load the sample material contained the fine powder of *Senna alata (L.) roxb* (20.0g) into the thimble. Then, the ethanol (300 ml) of 80% was added in Soxhlet apparatus a round of bottom flask then added into the thimble then placed it on the main chamber of the Soxhlet extractor and place into a heating mantle (Gritsanapan & Mangmeesri, 2009). Lastly attached the Soxhlet extractor above the round bottom flask and also attached the reflux condenser over the extractor, with cold water entered at the bottom and existed above and leave about 6 hours.

After 6 hours of extract *Senna alata (L.) roxb* by used Soxhlet extractor, put the extract into the 250ml of reagent bottle. To remove the ethanol is by using rotary evaporator, as extraction solvents, water distillate and ethanol (at a concentration of 95%) will be used. Twenty grams of finely grinded *Senna alata (L.) roxb* was weighting into a conical flask contained one hundred millilitres of the desire solvent (in this case, ethanol, and water), and the mixture were heated to room temperature (30 degrees Celsius) with vigorous glass stirred. It took one hour for the mixture to settle in a water reservoir heated to 40 degrees Celsius, 60 degrees Celsius, and 80 degrees Celsius. Afterward, the extracts will filter through Whatman no. 1 filter paper. To obtain crude extracts, the residual solvent will evaporate at 45 °C and 65 rpm in a rotary evaporator. The extracts were stored in the refrigerator at 4°C for subsequent use. And save leftovers of extraction into borosilicate glass with cap and ethanol (Lim et al., 2020).

3.2.2 PREPARATION OF PECTIN AEROGEL

To create pectin aerogels, the preceding stages of dissolving, gelation (in some cases, no gelation occur), solvent exchange, and drying with Supercritical drying machine. Pectin aqueous solutions are produced by dissolving pectin flour in distilled water at 65 °C while agitating at 400 rpm. Unless otherwise specified, pectin concentrations are typically reported as a weight percent (wt.%). In some cases, sodium ($CaCl_2$) will be added to pectin solutions while they will be stirring and distributing into Specimen containers (Groult et al., 2021).

Pectin solutions will be let to rest for 24 h at chiller. Depending on the conditions (concentration, and presence of extraction) solutions are gelling or not as determined via the vial tilting test (solution flowing or not). After 24 hours at the chiller, pectin solution was getting the gelling solution by used Calcium chloride ($CaCl_2$) to crate the thickness of gelling pectin solution and some extraction of *Senna alata (L.) roxb* was added into the solution with different (wt.%) of extraction. Pour all the sample into the sample containers after that put all the sample inside the -80°C freezer for 24 hours and for the next 3 days were used to performed supercritical drying to form the pectin aerogels. Two different weight percentages (%) of pectin aerogels were made which is 5wt% and 10wt%. The final dimensions of drying samples shrinkage inside samples bottles which in turn is controlled by pectin concentration and state of matter and the presence of $CaCl_2$.

Table 3.1. difference of concentration pectin and *Senna alata (L.) roxb* extraction

Sample	Concentration Pectin (wt.%)	*Senna alata (L.) roxb* extraction (%)
PA 1	5	0
PA 2	5	1
PA 3	5	1.5
PA 4	5	2
PA 5	10	0
PA 6	10	1
PA 7	10	1.5
PA 8	10	2

3.2.3 CHARACTERIZATION OF AEROGELS

3.2.3.1 MORPHOLOGY ANALYSIS

Scanning electron microscopy, or SEM, is a high-powered microscope that uses electron beams to scan a material and provide an image of excellent quality. Several steps are required to use a scanning electron microscope (SEM), such as preparing the sample for imaging, choosing an appropriate objective aperture, and raising the magnification. The SEM used a focused beam of high-energy electrons to visualize the surface of the sample and generate various signals, allowing for the qualitative and quantitative determination of chemical compositions, crystal orientations, and structural details. Using a JSM IT200 InTouchScope SEM, researchers at Universiti Malaysia Kelantan conducted the analysis.

3.2.3.2 FTIR ANALYSIS

FTIR stands for Fourier Transform Infrared Spectroscopy. It is a way to analyse and identify organic, polymeric, and sometimes inorganic materials. FTIR spectrometers need samples to be prepared first, and one common way to do this is to use potassium bromide (KBr) to turn solid samples into clear pellets. The device shines infrared light through the sample. Some of the light is absorbed, but some is also passed through. Researchers at Universiti Malaysia Kelantan used the Nicolet iS50 model to do this evaluation.

3.2.3.3 TGA ANALYSIS

TGA determines the thermal stability of compounds at high temperatures. Thermogravimetric Analysis (TGA) requires controlled heating of a sample and observation of weight loss over time or temperature. The weight reduction after furnace heating and crucible insertion is measured with a balance. The material under study affects the temperature range of 25°C to 600°C and heating rates of 20 °C/min and gas flow of nitrogen atmosphere with a rate of 50 ml min^{-1}. Materials science use TGA to determine material composition, purity, and heat stability. Universiti Malaysia Kelantan used EW-25753-44 Mettler Toledo TGA2 Thermogravimetric Analyzer with Small Furnace, XP1 Balance, and TGA Sensor.

3.2.3.4 ANTIMICROBIAL TESTING

The disk diffusion method is one the technique used to determine the susceptibility od pathogenic bacteria to antimicrobial agents. By streaking the microbial of E-coli on agar plate then incubate for 24 hours. Use a new agar plate then strike with cotton bud that absorb E-coli on the agar plate and then drop the antibacterial and E-coli on the agar and put the sample as it divided into 4 sections. Put the sample and incubate for 24 hours to see the clear zones and then measured the clear zones to see the result of the samples.

RESEARCH FLOWCHART

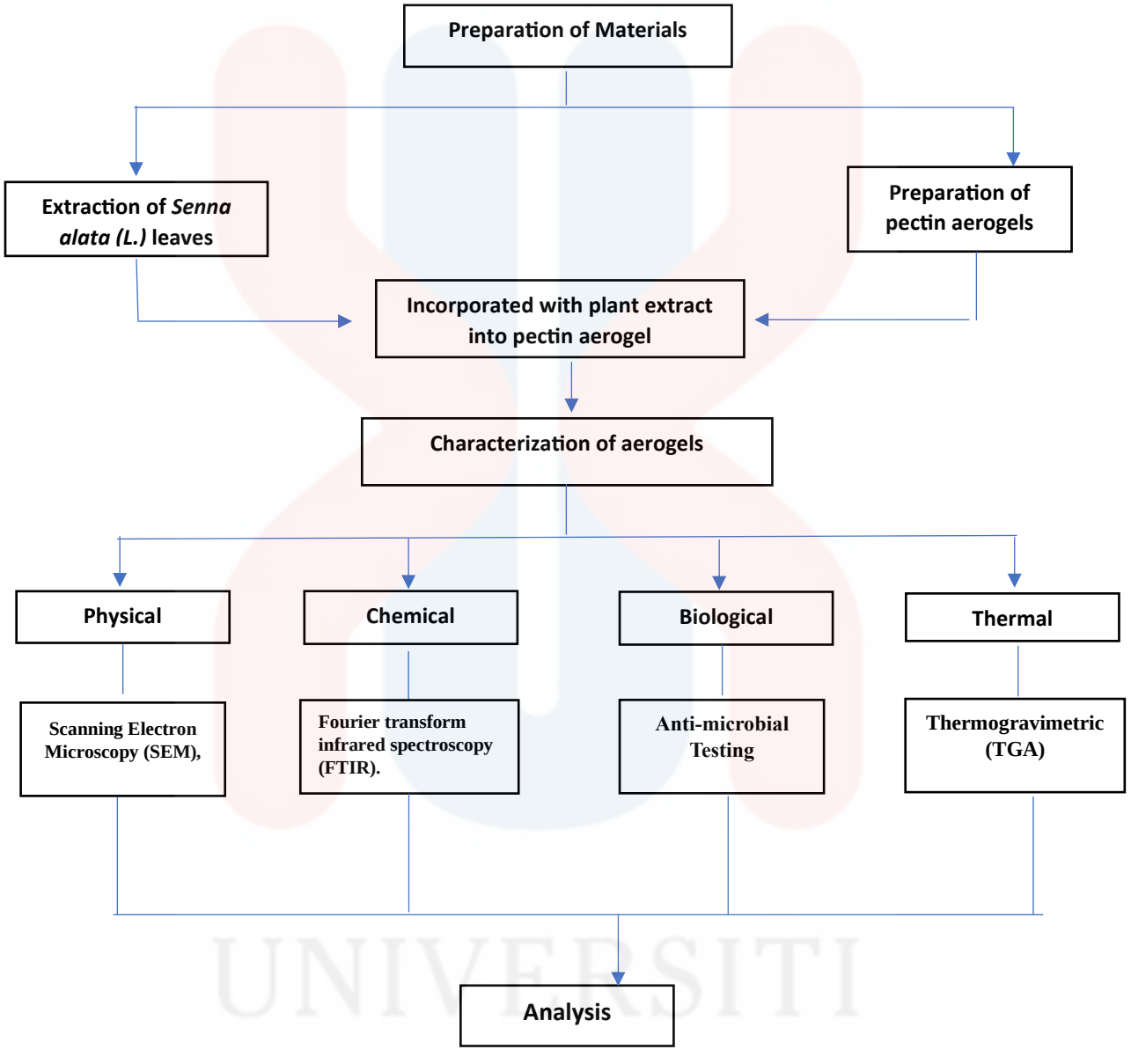

Figure 4: Flowchart of Pectin Aerogels.

CHAPTER 4

RESULTS & DISCUSSIONS

The characterization of the samples that are the pectin aerogels (5 wt. % and 10 wt. %) with pure and different extraction of *Senna alata (L.) roxb* were analyses by scanning electron microscopy (SEM), fourier transform infrared analysis (FTIR), thermogravimetric analysis (TGA) and antimicrobial analysis. *Senna alata (L.) roxb* was obtained through the extraction by using Soxhlet extraction with the ethanol that was loaded into pectin aerogels in (5 wt. % and 10 wt. %) with different concentrations. Thus, can search about the contribute to absorbency, compatibility, and antibacterial potential, this combination appears promising for food packaging absorbent cushioning.

4.1 SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

Scanning Electron Microscopy (SEM), it. The pectin core will be covered by *Senna Alata (L.) roxb*, as seen in SEM images at a scale of 100 to 250 microns. Each of the two layers is permeable. In this analysis we can see the porosity of the pectin aerogel and morphology, also structure is highly porous, having a complex interconnecting network of pores. And (PA 5 wt. %) samples were examined 100x-250x magnification. At (figure 4.1 (A)), Pa 5 wt. % pure appeared as rough surface without any porous were appeared because the pectin does not have any mixture of extraction. Meanwhile, for (figure 4.1 (B)), Pa 5 wt.% 1% of extraction was examined as the porous appeared multiples on the surface of the pectin aerogels. It is because the crosslinking of pectin aerogels with *Senna alata (L.) roxb* and calcium chloride ($CaCl_2$) happened and consisted of 1% of extraction with 90ml of distilled water.

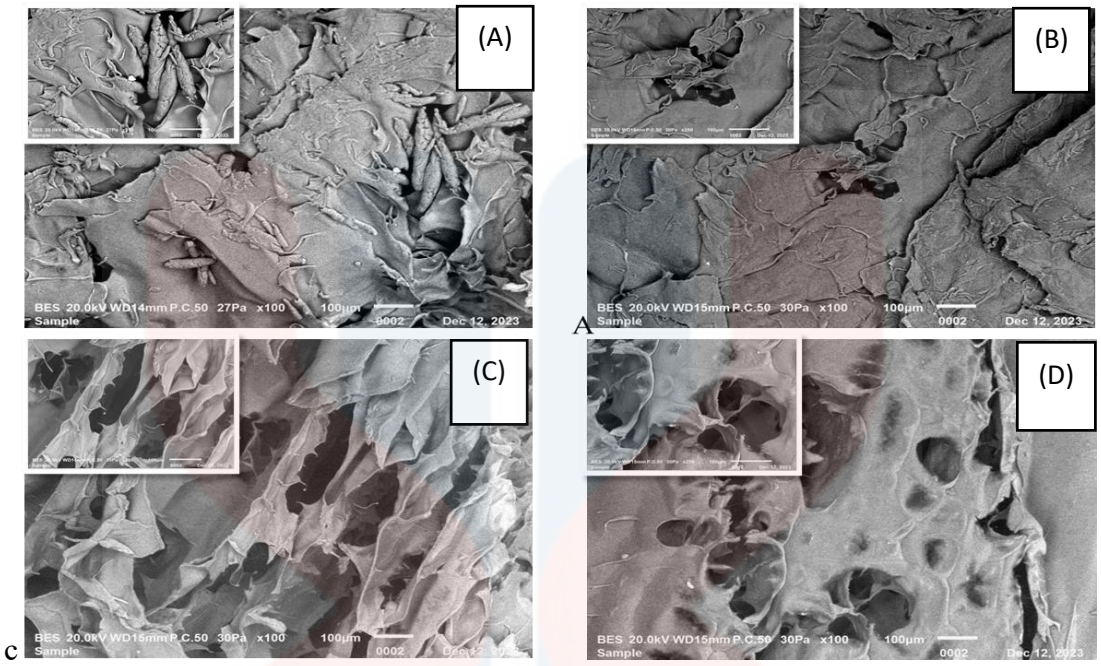

Figure 4.1. SEM images of Pectin Aerogels 5wt% (A) PA 5wt% PURE (B) PA 5wt% 1% (C) PA 5wt% 1.5% (D) PA 5wt% 2% at 100x and 250x magnificent (100x and 250x)

Furthermore, in (Figure 4.1 (C)) PA 5wt% 1.5% was examined, and the results shown that there a lot of morphology and porosity of the pectin aerogels has appeared on the surface because the crosslinking between pectin and *Senna alata (L.) roxb* with calcium chloride was successfully to obtain the porosity with the 1.5% of extraction with 90ml of distilled water. Meanwhile, in (Figure 4.1 (D)) PA 5wt% 2% shown the result the porosity of the samples can be seen clearly as the porosity appeared on the surface because of the highest percentage of extraction from *Senna alata (L.) roxb* extraction that been crosslinking with the distilled water, pectin, and calcium chloride.

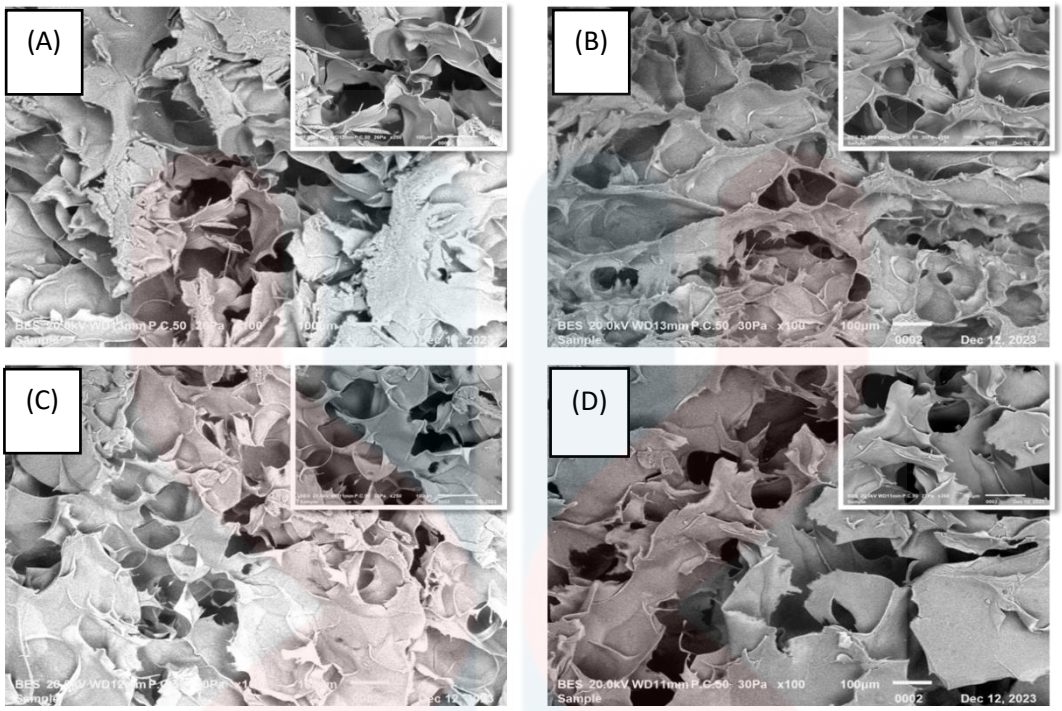

Figure 4.2. SEM images of Pectin Aerogels 10wt% (A) PA 10 wt.% PURE (B) PA 10 wt.% 1% (C) PA 10 wt.% 1.5% (D) PA 10wt% 2% (100x and 250x)

In (Figure 4.2 (A)) PA 10wt% pure, the image above shows that the porosity does not clearly appear on the surface and seems roughly surface as it that pectin is the pure one which is there doesn't have any extraction that crosslinking with distilled water, pectin, and calcium chloride. So, there will be no porosity in the pure pectin aerogels. Thus, in (Figure 4.2 (B)) PA 10wt% 1% shown that a little porosity appeared on the surface of the aerogels as it confirmed that the aerogels are crosslinking with the 1% *Senna alata (L.) roxb* extraction with distilled water, pectin, and calcium chloride. Meanwhile, for the (Figure 4.2 (C)) shown that multiples porosity appeared clearly and in good shape as the extraction that used is 1.5% with the crosslinking of distilled water, pectin, and calcium chloride. And lastly, in (Figure 4.2 (D)) shown that a lot of porosity clearly can be seen and deeper the porosity because the highest extraction is used with 2% of extractions that crosslinking with distilled water, pectin, and calcium chloride.

4.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

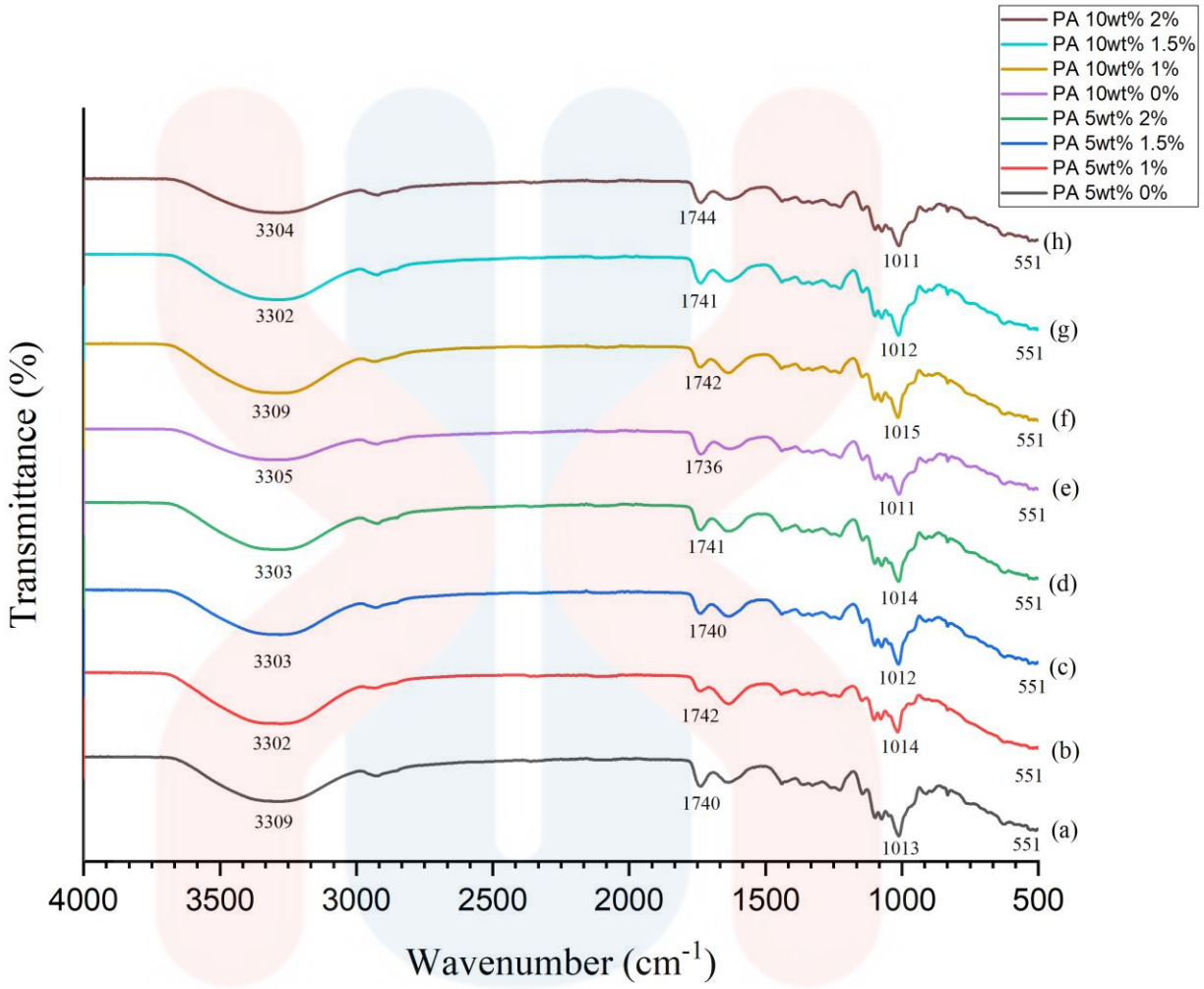

Figure 4.3. Fourier transform infrared spectroscopy (FTIR) spectra of pectin aerogels with different weight percentages**.**

FTIR spectra of pectin aerogels with different weight percentages are shown in Figure 4.3. For the curve (a), the broad absorption band located at wavenumber 1013 cm^{-1} indicated to the characterization of pectin aerogels bond (C-O) stretching vibrations. (C-O) stretching peaks are usually intense and between wavenumbers 1300 and 1000 cm^{-1}. Meanwhile for the peak at between 3330 to 3309 cm^{-1} the bond is primary aliphatic alcohols (-OH) which is the frequencies stretching is 3643 to 3630 cm^{-1}.

Moreover, for the curve (b) the stretching bond that appeared is (S=O) as the absorption at the peak stretch in the range of 970-1070 cm^{-1}, any chemical compound belonging to the organic class in which the atoms are connected by single, double, or triple bonds to form nonaromatic structures. Sulphur is doubly linked to oxygen and singly to the organic group. Meanwhile for the peak at between 3330 to 3309 cm^{-1} the bond is primary aliphatic alcohols (-OH) which is the frequencies stretching is 3643 to 3630 cm^{-1}. The infrared peaks of alcohols are broadened because of hydrogen bending and are hence easy to spot.

Besides, all the sample like the curve (c) and (d) are shown the stretching bond that appears is C=O and (C-O). Both the samples have exactly same bond at the peak around wavenumbers, typically observed in the range of 1740-1735 cm^{-1}. The (C=O) stretch, which is associated with the carbonyl group in the ester (C-O) are formed by the reaction between a carboxylic acid and an alcohol, resulting in the elimination of water at the range of 1300-1000 $cm^{-1.}$ Chemical bonding of aliphatic sulfoxides by analysing the characteristic absorption bands associated with the (S=O) stretch in the range of 970-1070 cm^{-1}. The bond is primary aliphatic alcohols (-OH) which is the frequencies stretching is 3643 to 3630 cm^{-1}.

Next, for the curve (e) The FTIR spectrum of ketones would show a characteristic absorption band associated with the (C=O) stretch, which is typically observed in the range of 1640-1815cm^{-1}. This absorption band is associated with the carbonyl group in the ketone functional group. The presence of monosubstituted alkynes can be identified by the characteristic (=C-H) out-of-plane bending absorptions in the 700 to 1000 cm^{-1}. Aliphatic hydrocarbons typically exhibit (C-H) stretching vibrations in the range of 3000-2800 cm^{-1}, which are associated with the hybridized carbon atoms in the aliphatic chain. The intensity and position of these absorption bands can provide information about the degree of branching and the length of the aliphatic chain.

Besides, absorption bands associated with the (S=O) stretch in the range of wavenumbers between 970-1070 cm^{-1} at the curve of (f) and (g). Its functional group can exhibit resonance, leading to some delocalization of electron density between the sulphur and oxygen atoms. This delocalization contributes to the stability of the sulfoxide. Lastly, at the wavenumbers at 3302-3309 cm^{-1} the bonding (C-O) Vibration has a high value due to the dipole moment being pushed and pulled, resulting in powerful peaks. This (C=O) stretch is typically observed in the range of 1740-1735 cm^{-1} which is associated with the carbonyl group in the ester functional group.

Lastly, for the curve (h) the broad absorption band located at wavenumber 1013 cm^{-1} indicated to the characterization of pectin aerogels bond (C-O) stretching vibrations. (C-O) stretching peaks are usually intense and between wavenumbers 1300 and 1000 cm^{-1}. Meanwhile for the peak at between 3330 to 3309 cm^{-1} the bond is primary aliphatic alcohols (-OH) which is the frequencies stretching is 3643 to 3630 cm^{-1}.

4.3 THERMAGOMETRIC ANALYSIS (TGA)

TGA is continuous process, involving the measurement of sample weight in accordance with increasing temperature in the form of programmed heating. The thermal degradation behaviours of pectin aerogels pure and with extraction were studied in the range 25-600°C under nitrogen atmosphere. (Ye et al., 2018). Figure 4.4 (a), (b), (c), and (d) showed the thermogravimetric analysis (TGA) of 5wt% pure, 5wt% 2%, 10wt% pure, and also 10wt% 2% respectively.

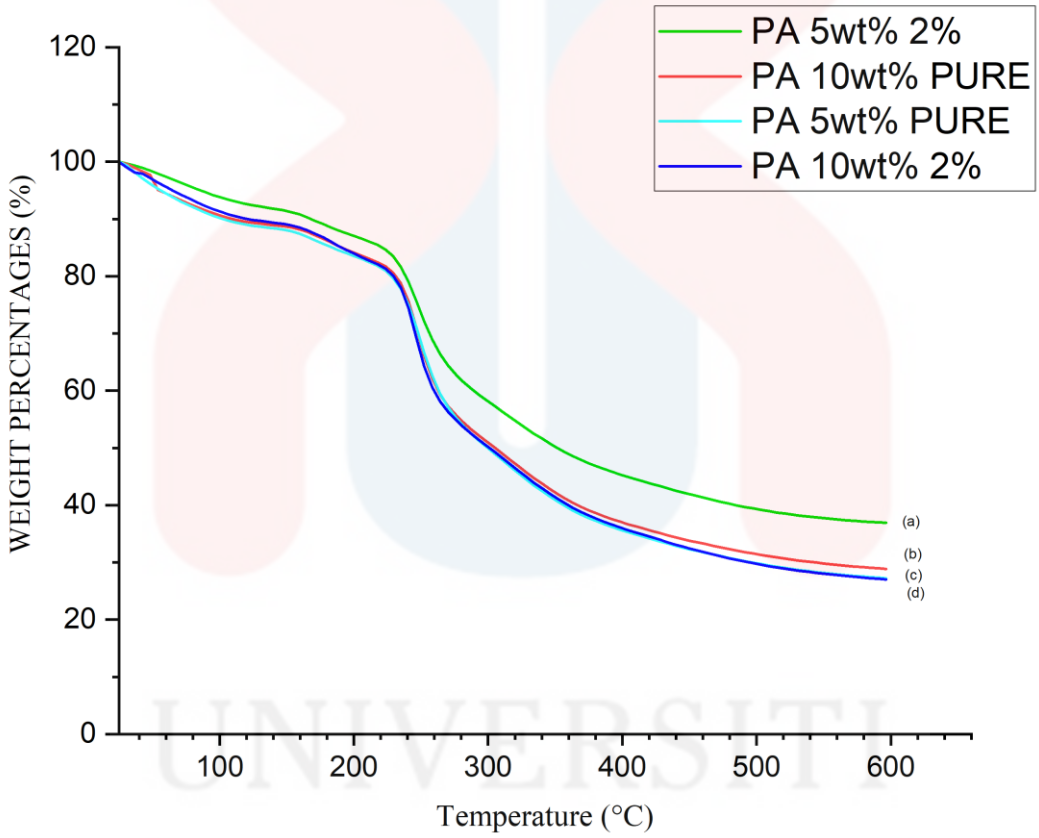

Figure 4.4. TGA (a) PA 5wt% 2% (b) PA 10wt% PURE (c) PA 5wt% PURE (d) PA 10wt% 2%

In Figure 4,4, all of the samples had a small amount of weight loss at 100 °C, which was caused by water that was weakly attached to the surfaces of the pectin aerogels evaporating. All of the curves of the samples showed a single, interesting breakdown that was linked to the weight loss.

At the relatively narrow temperature range (100 °C to 240 °C) the weight loss and corresponding derivative weight curves of the pectin aerogels (a), (b), (c), and (d) are demonstrated and it shown that the mass loss was supposed to be the vaporization of water from the sample (M. Alnaief et al., 2012). Curve (c) had earliest weight loss other than other curves from 100 wt.% and loss around 80wt.%.

The second weight loss were during the range temperature around (240 °C to 280 °C), the curve (d) had earlier weight loss than other curves. The degradation behaviour is attributed to the breakdown of the pectin structure under the influence of increasing temperature as the curve (b), (c), and (d) indicating a multi-stage decomposition process.

And lastly, the last weight loss during range temperature (280 °C to 500 °C) may have been due to the thermal decomposition. And the result shown that the first and second weight loss, the curve of (a), (b), (c), and (d) shown the best thermal stability. It could the fast cross-linking that could make the loss mass of pectin aerogels happen.

4.4 ANTIMICROBIAL ANALYSIS

The antibacterials activities of prepared pectin aerogels were studied by using the disc diffusion method. The antibacterial method capacity was determined by measuring the diameter of the clear zone of the inhibition around the samples after 24 hours in room temperature. The picture below shown in (figure 4.2.1).

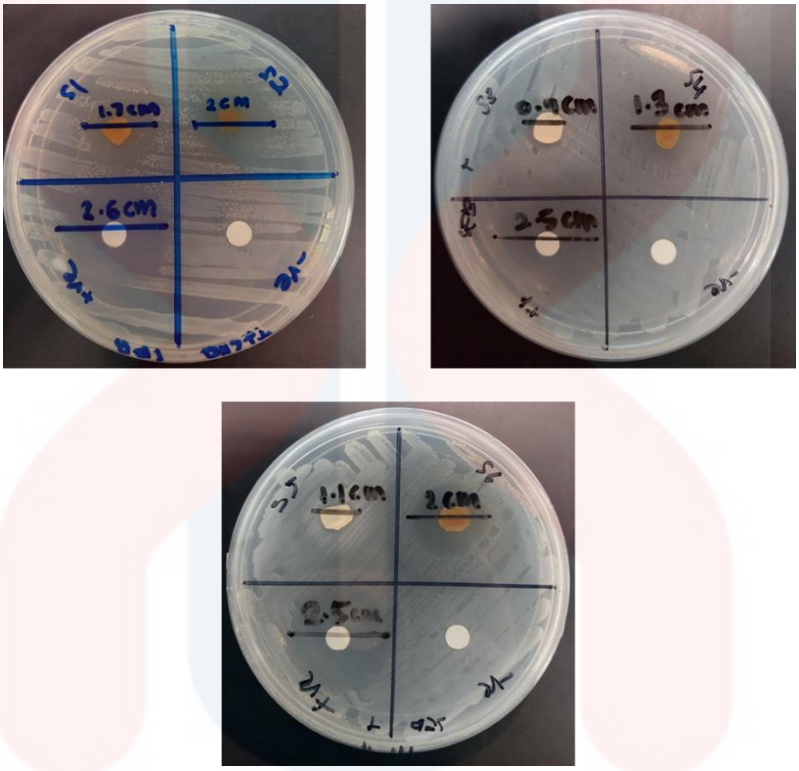

Figure 4.5. Optical images of clear zones of pectin aerogels with E-coli. (S1) PA 5wt% 2%, (S2) PA 10wt% 2%, (S3) PA 5wt% pure (S4) PA 5wt% 1.5% (S5) PA 10wt% pure (S6) PA 10wt% 1.5%

In the first picture of the sample of (S1) and (S2) shown the clear zone of each of the samples from PA 5wt% 2% and PA 10wt% 2% in diameter of clear zone for (S1) is 1.7cm. Meanwhile the (S2) diameter of the clear zone is 2cm. And as the negative (-) zones also show clear zone around 2.6cm because the zone contains the antibacterial solution which is Chloramphenicol and positive (+) zones contained distilled water.

For the image of the second sample of (S3) and (S4) shown that the clear zone of each sample from PA 5wt% pure and PA 5wt% 1.5% in diameter of clear zone for (S3) is 0.9cm and for (S4) is 1.3cm. And as the negative (-) zones also shown clear zone around 2.6cm because the zone contains the antibacterial solution which is Chloramphenicol and positive (+) zones contained distilled water.

Lastly the sample of (S5) and (S6) shown that the clear zones appeared clearly on the disc surface from PA 10wt% pure and PA 10wt% 1.5% in diameter of clear zone for (S5) is 1.1cm and (S6) is 2cm. And as the negative (-) zones also shown clear zone around 2.6cm because the zone contains the antibacterial solution which is Chloramphenicol and positive (+) zones contained distilled water.

With this antimicrobial analysis, we can see that the pectin aerogels with 5wt% to 10wt% differences could be seen clearly the clear zone that pectin aerogels are proven from previous study James Doughari Hamuel & Okafor, (2008) that all the extractions demonstrated significant activity against gram positive bacteria and fungi. From the samples of PA 5wt% pure, 1.5% and 2% can be seen the increasing of the clear zone from o.4cm, 1.3cm to 2cm against the E-coli. And also, from the PA 10wt% pure, 1.5% and 2% can be seen the increasing of the clear zone also from the 1.1cm, 2cm to 2cm each sample against the E-coli. The present study clearly illustrates that the prepared pectin aerogels with *Senna alata (L.) roxb* extractions show very excellent antibacterial results.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, pectin aerogels loaded with *Senna Alata (L.) roxb*. leaf extracts were successfully prepared with 5wt% and 10wt% extract concentrations. Characterization using SEM, FTIR, TGA and antimicrobial testing showed that incorporation of the plant extract enhanced the properties of the aerogel.

The SEM analysis demonstrated increased porosity in the aerogel microstructure with higher percentages of extract loading. FTIR confirmed the presence of functional groups from *Senna alata (L.) roxb* as well as pectin. TGA results indicated good thermal stability of the loaded aerogels. Finally, antimicrobial tests showed significant antibacterial activity against E. coli, confirming the extracts preserved their bioactive properties in the aerogel matrix.

Overall, it has proven that pectin aerogels can be an effective carrier of *Senna alata (L.) roxb* extracts to produce sustainable, biodegradable, and high-performance absorbent pads for food packaging applications. The 10wt% loaded aerogel displayed slightly better properties and is a promising formulation. Further research can build on these positive results for commercialization of these bio-based aerogels to replace conventional petroleum-based products. Their biocompatibility, absorbency and antimicrobial features make them an eco-friendly “green” alternative for food packaging industries.

For the recommendation, further optimize the preparation parameters such as pectin concentration, drying method, and extract composition to tailor the loaded aerogel properties for specific food packaging requirements. Also, can conduct more advanced characterization such as surface area analysis, mechanical testing, and cytotoxicity assays to fully validate the aerogel performance.

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APPENDICES A

A1: *Senna alata (L.) roxb* powder.

A2: Soxhlet Extraction of Senna Alata (L.) *roxb.*

A3: Extraction of Senna Alata (L.) *roxb*

A4: Sample of 5wt% and 10wt% pectin aerogels

A5: Pectin aerogels 5wt%

A6: Pectin aerogels 10wt%