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Original Article

# The Potential of Lai Fruit Seed Starch (*Durio kutejensis*) as A New Excipient in Pharmaceutical Preparations through Specific and Non-Specific Characterization

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**Abstract**

**Background**: Lai fruit seeds (*Durio kutejensis)* contain starch. In the pharmaceutical field, starch can be used as an ingredient in the formulation of tablet preparations such as fillers, binders, and disintegrants. Objective: This research aims to determine the potential of Lai Fruit Seed Starch (*Durio kutejensis*) as a new excipient in Pharmaceutical Preparations. **Methods**: The research process started with plant determination, sample collection, starch manufacture, and examination of specific and non-specific characteristics, as well as testing the starch content of other fruit seeds. **Result**: The content of the extract is 12.5% water soluble, and the content of the extract is soluble in ethanol, 1.5%. Based on the non-specific parameters of lai fruit seed starch, the water content was 11.4%, the ash content was 1.5%, and the acid insoluble ash content was 0.75%. The starch content test of lai fruit seeds was found to contain fructose. **Conclusion**: The seed starch of lai fruit has the potential to be used as an alternative to new excipients in pharmaceutical preparations**.**

***Keywords:*** *characteristics, starch, lai fruit seeds (Durio kutejensis)*

## Introduction

Indonesia's forests have the highest biodiversity globally, including the diversity of tropical forest fruit species. One type of forest plant with potential as a food source is Durio. Kalimantan is the centre of Durio diversity in the world because it has a wealth of species, genetic resources, and ecosystems that allow Durio to thrive (Priyanti,2012). The lai plant (*Durio kutejensis*) is the durian plant that grows in the tropics. The advantage of lai fruit is its high levels of vitamin A. This can be seen in the very yellow colour of the fruit flesh, which contains carotene, a provitamin A (Antarlina, 2016). Some of the uses of Durio by the Dayak community are that the young shoots and fruit can be used as fresh vegetables. The flesh, which is usually eaten fresh, can also be processed into durian lunkhead, or tempoyak, which can also be eaten(Priyanti,2012).

Research has been carried out on several types of *Durio*. including a study conducted by Amir and Saleh (2014) stating that durian fruit seeds (*Durio zibethinus*) have secondary metabolites in the form of alkaloids, flavonoids, and triterpenoids and have antioxidant activity. Durians seed flour can be produced as a substitute for wheat flour, and durian seed starch can be used as a tablet mixture.

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*Durio Dulcis* seeds have a water content of 4.63%, an ash content of 2.22%, and secondary metabolites in the form of alkaloids (Susi, 2017).

Suryani and Zaharah (2017) discovered that lai plants have a huge potential for discovering and developing secondary metabolites that can be used as a treatment (Suryani & Zaharah, 2017). Several studies related to other plants, such as lai flower water decoction, can treat heartburn or fever (Priyanti, 2012), and lai fruit peel extract is antibacterial against *Staphylococcus aureus* and *Salmonella enterica* serovar *typhi* bacteria (Muhsin & Sudrajat, 2016). Lai fruit extract has antioxidant properties with the potential to treat hyperpigmentation and skin lightening (Arung, *et.al*,. 2015).

Starch is a carbohydrate that is a glucose polymer and consists of amylose and amylopectin (Jacobs & Delcour, 1998). In the pharmaceutical field, starch can be used as an additional ingredient in the formulation of pharmaceutical preparations, such as fillers, binders, or disintegrants in tablet preparations (Gunawan & Mulyani, 2004). The main components of the seeds of the Durio family are starch and protein (Brown, 1997). Based on this, a study was conducted on the characterization of lai fruit seed starch to be used as initial data in the development of pharmaceutical preparations to increase the economic value of lai fruit seed waste and increase the availability of medicinal additives from natural ingredients.

## Materials and Methods

### *Tools*

The tools needed in this research are a set of glassware, an analytical balance, a water bath, a blender, metal spatula, porcelain cup, porcelain exchanger, furnace, horn spoon, oven, silica gel plate GF254, a spirit lamp, ash-free filter paper, mesh sieve: 80, pH meter, desiccator, and microscope.

### *Materials*

The materials needed in this study were lai fruit seed starch, aqua dest, chloroform, 95% ethanol, dilute HCl, n-butanol, acetic acid, glucose, sucrose, fructose, lactose, Fehling's reagent A, Fehling's reagent B, iodine reagent, concentrated $H_2SO_4$, and naphthol solution.

### *Research design*

The research is non-experimental, namely a study in which observations are made on several variables according to what they are, namely about the characteristics of the starch of other fruit seeds. The research stages started by determining other plants, collecting raw materials, and making starch. A characterization was carried out, which included organoleptic examination, microscopic examination, determination of water content, determination of water-soluble extract content, determination of ethanol-soluble extract content, determination of total ash content, determination of acid-insoluble ash content, carbohydrate test, polysaccharide test, reducing sugar test, and thin layer chromatography profile.

### *Plant Determination*

The determination of other plants was carried out at the Laboratory of Plant Anatomy and Systematics, Faculty of Mathematics and Natural Sciences, University of Mulawarman Samarinda.

### *Sample collection*

The samples were lai fruit seeds obtained on Jalan Batu Cermin, North Samarinda District, East Kalimantan.

### *Starch Making*

Wet sorting is used to separate the meat from the fruit seeds after they have been harvested. The seed coat was peeled and weighed, then washed and soaked for 2 hours. The grains are then mashed to form coarse slurry with the addition of 1/3 of the weight of aqua diet, stirred, and then filtered with a cloth, squeezed until all the water runs out. The dregs are remixed with 1/3 of the aqua dest, stirred, then pressed again until the water runs out. Repeat until you get clear juice. The resulting suspension was then deposited for 24 hours and then decanted. The product of decantation is called starch. Wet starch is dried in an oven at 500 °C or dried in direct sunlight to dry, and the dried starch is then sieved with an 80-mesh sieve. The resulting starch is then calculated as the yield (Suheri, *et.al*.2015).

Yield (%) = weight of sediment (g)/weight of other seeds (g) x 100%

***Specific Characteristics Check***

*Organoleptic Examination*

The organoleptic examination aims to determine the characteristics of lai fruit seed starch powder by observing the shape, color, smell, and taste of the starch directly.

*Microscopic Examination*

The examination was carried out by placing a small amount of lai fruit seed starch on a glass object and then dripping with aqua dest. The fragments were observed under a microscope to identify fragments in the shape and characteristics of starch grains in starch powder of other fruit seeds.

*Determination of Water-Soluble Extract Levels*

A total of 5 grammes of starch powder were macerated for 24 hours with 100 mL of chloroform water (2.5 mL of chloroform in 100 mL of distilled water) using a clogged flask while occasionally shaking for the first 6 hours and then allowed to stand for 18 hours. As much as 20 ml of the filtrate is evaporated in a shallow dish on an average basis, which has been tarred in a water bath to dry; the filtrate is heated at 1050C, then cooled, and weighed until a constant weight is obtained. Calculate the content in percent water-soluble juice calculated against the material that has been dried in the air.

Water soluble juice content = (Earth weight)/(Weight of lai fruit seed starch) x (100/20) x 100%

*Determination of Soluble Essence Levels in Ethanol*

A total of 5 grams of starch powder was macerated for 24 hours with 100 mL of 95% ethanol using a clogged flask, occasionally shaking for the first 6 hours and then allowing it to stand for 18 hours. Filter quickly to avoid ethanol evaporation. A total of 20 ml of the filtrate was evaporated in a shallow dish on an average basis, which had been tarred in a water bath to dry, and the filtrate was heated at $105^0$C, then cooled, and then weighed until a constant weight was obtained. Calculate the content in percent ethanol-soluble essence (95%), calculated against the material that has been dried in the air.

Essence content dissolved in ethanol = (Earth weight)/(Weight of lai fruit seed starch) x (100/20) x 100%

***Non-specific parameters Check***

*Determination of Water Content*

A total of 5 grams of starch powder was weighed in a shallow dish based on a known average weight, then dried in an oven at $105^0$C for 30 minutes, cooled in a desiccators, and weighed to a constant weight.

Moisture content by (b-(c-a))/b x 100%)

Description:

a. instead of the word by Weight of dry cup constant
b. instead of the word by Weight of initial sample
c. instead of the word by Weight of cup and sample (Andarwulan, *et.al*. 2014)

*Determination of Total Ash Content*

A total of 2 grams of starch powder was put in a porcelain crucible that had been ignited and leveled, leveled. The crucible was incandescent slowly until the charcoal ran out; incandescent was carried out at a temperature of $600^0$C for 3 hours, then cooled and weighed until a constant weight was obtained. Add a hot water filter through ash-free filter paper if the charcoal does not disappear. Burn the residue and filter paper in the same crucible. Put the filtrate into a crucible, evaporate. Incandescent until the weight remains, weighed. Ash content is calculated on the material that has been dried in the air.

Total ash content = (weight of residual incandescent ash)/(Weight of other fruit seed starch) x 100%

*Determination of Acid Insoluble Ash Content*

The ash obtained from the determination of the ash content was boiled with 25 mL of dilute hydrochloric acid for 5 minutes. The acid-insoluble part was collected, filtered with ash-free filter paper of known weight, washed with hot water and then ignited, then cooled and weighed until a constant weight was obtained. The acid-insoluble ash content is calculated on the material that has been dried in the air.

Ash content is not soluble in acid = (Weight of incandescent ash)/(Weight of other fruit seed starch) x 100% (Depkes RI, 1995)

*Chemical Content Test*

Before the content test, a starch test solution was prepared. The sample in the form of lai fruit seed starch was made into a test solution by weighing 5 g of starch, dissolved in 100 mL of distilled water, and adding HCl until the pH was around 1, then heating and filtering. The filtrate results are put into a bottle and labeled with the test solution (Kusbandari, 2015).

*Carbohydrate Test*

Put 2 mL of starch test solution into a test tube, add five drops of 3% naphthol solution in ethanol, add 2 mL of concentrated $H_2SO_4$ and flow carefully through the edge of the tube. A purple ring is formed (Autherhoff, 1987).

*Polysaccharide Test*

Put three drops of starch solution into a test tube, then add two drops of iodine solution and observe the changes (Setyaningrum, 2018).

*Reducing Sugar Test*

A total of 10 drops of Fehling's reagent A and ten drops of Fehling's reagent B were mixed, then added to 3 ml of the test solution that had been put into a test tube. The test tube was heated and then observed; it was positive if a brick-red precipitate was formed (Juwita, *et.al*. 2013).

*Thin Layer Chromatography Profile*

The eluent utilised in this work was a mixture of n-butanol, acetic acid, and aqua dest in a 12:6:6 ratio (Wibisono, 2014), which was subsequently saturated in the chamber. A 10% glucose solution, 10% sucrose, 10% fructose, and 10% lactose solution were employed as the comparator solutions in this investigation. The starch test solution and the reference solution were spotted on a silica gel GF254 plate activated by heating at $110^0$C for 15 minutes in the oven. In the chamber, the ready plate is inserted. The scale is developed until the eluted component's motion reaches the limit mark, after which the plate is dried and the Rf value of the eluted component is measured.

Rf = (spot distance)/(distance traveled by eluent)

*Data analysis*

The data analysis method used in this research is descriptive in qualitative and quantitative data. Qualitative data includes the results of organoleptic observations; microscopic observations; carbohydrate tests; polysaccharide tests; and reducing sugar tests. Quantitative data consists of the results of the determination of water content; determination of water-soluble extract content; determination of ethanol-soluble extract content; determination of total ash content; determination of acid-insoluble ash content; and thin-layer chromatography profile.

## Results and Discussion

The results reveal that the plant under investigation is the one that the researcher had in mind. This is in line with the goal of the activity, which is to discover the truth about a plant that is actually another plant (*Durio kutejensis*). Table 1 shows the findings of the investigation.

**Table 1.** Classification of *Durio kutejensis*

| **Classification** | ***Durio kutejensis*** |
|---|---|
| Kingdom | Plantae |
| Subkingdom | Viridiplantae |
| Super Division | Embryophyta |
| Division | Tracheophyta |
| Class | Magnoliopsida |
| Ordo | Malvales |
| Family | Bombacaceae |
| Genus | Durio |
| Spesies | *Durio kutejensis* |

The samples selected and taken were ripe lai fruit to ensure that the seeds contained more starch. Starch content generally increases with increasing harvest age; the older the harvest age (ripe fruit), the higher the starch content because more starch granules are formed (Nurdjanah, *et.al.* 2012). Subsequently, the starch yield was calculated, and the result was 19.6%. The small yield produced can be due to the presence of starch lost during the starch manufacturing process or the lack of starch content in the plant. The quality of the final starch yield is heavily influenced by extraction in the starch production process (Rahman, *et.al*. 2015). The process of extracting and draining starch will also result in reduced starch weight loss so that it will affect the yield of starch (Irhami & Kemalawaty, 2019).

### Specific Characteristics of Lai. Fruit Seed Starch

#### *Organoleptic Examination*

The organoleptic examination was carried out to identify the characteristics of starch by describing the morphology in the form of shape, color, smell, and taste using the five senses. The results of the examination can be seen in table 2.

**Table 2.** Results of organoleptic examination of other fruit seed starch

| **Parameter** | **Result** |
|---|---|
| Form | Fine powder |
| Color | Brownish white |
| Smell | Typical |
| Flavor | no taste |

According to research conducted by Priyanti (2012), who indicates that the starch of durian fruit seeds has a fine powder form and a brownish white hue, the morphology of the shape and colour of the seed starch of lai fruits is comparable to the starch of durian fruit seeds. This is due to the fact that they are nonetheless classified as members of the same genus.

***Microscopic Examination***

Microscopic examination is a type of examination that uses a microscope with a camera and magnification that may be adjusted as needed. The purpose of the microscopic investigation was to learn the features of starch so that it could be used to identify the plant by looking at the powder under a microscope (Wulandari, 2011). Figure 1 shows the findings of a microscopic investigation of lai fruit seed starch.

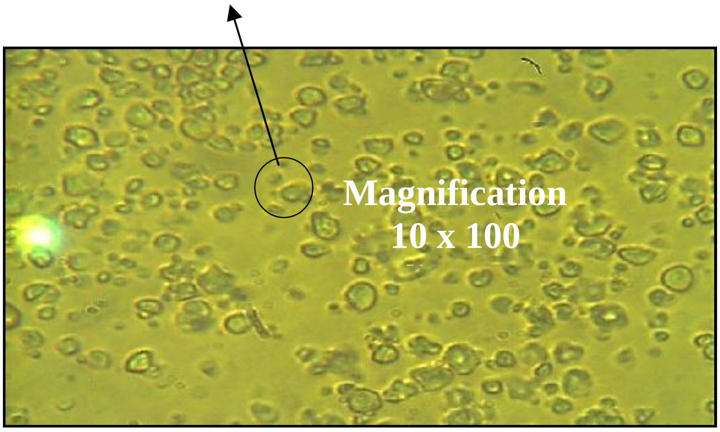


**Figure 1.** Results of microscopic examination of lai fruit seed starch

Water media is used so that starch grains can be seen because, based on the solubility of starch, it is not soluble in cold water so that starch grains can be seen (Depkes RI, 1979). Based on the results of starch microscopy examination of the seeds of lai fruit, it was found that the identified fragments of starch were single starch grains, the shape of the starch grains varied, and there was a hilus in the middle, which was in the form of a point. The results of microscopic examination of lai fruit seed starch also have similarities with durian seed starch based on research by Soebagio & Sriwododo (2009) that the identification fragment of durian seed starch is a single starch grain, slightly rounded and multi-faceted, the hilus is located in the middle, and the lamellae are not clearly visible.

***Water Soluble Extract and Ethanol Soluble Extract***

The water-soluble juice content test aims to determine the levels of polar water-soluble chemical compounds, while the ethanol-soluble essence test aims to determine the levels of ethanol-soluble chemical compounds, both polar and non-polar compounds contained in the sample, in this case, starch (starch) (Supriningrum, *et.al*. 2017). Adding chloroform to a test for water-soluble extracts using water as a solvent seeks to prevent bacteria from growing and interfering with the testing process. Meanwhile, because the ethanol solvent was already antibacterial, no chloroform was used in the ethanol-soluble extract test. The use of water and ethanol solvents is allowed because both are liquid solvents that are allowed and meet pharmaceutical requirements (Paramita, *et.al*. 2019). The results of water-soluble and ethanol-soluble extracts can be seen in table 3.

**Table 3.** Results of soluble extracts in water and in ethanol

| Description | Result |
|---|---|
| Water soluble juice content | 12.50% |
| Ethanol soluble juice content | 1.50% |

Based on the data obtained, it shows that the content of the juice attracted by ethanol is lower than that of water; This corresponds to Febrianti's findings, which show that the water soluble extract content in extracts and powders is larger (19.54 percent and 19.53 percent, respectively) than the ethanol soluble extract level (16.13 percent and 14.55 percent). It's probable that the sample contains more polar secondary metabolites than semi-polar secondary metabolites, making these chemicals more soluble in water than in ethanol (Febrianti, *et.al*. 2019).

**Non-Specific Characterization Results**

The examination results based on non-specific parameters can be seen in table 4.

**Table 4.** Results of water content, total ash content, and acid insoluble ash content

| Description | Result |
|---|---|
| Water content | 11.40% |
| Total ash content | 1.50% |
| Acid insoluble ash content | 0.75% |

Determination of water content aims to provide a minimum limit or range regarding the amount of water content in the sample (Depkes RI, 2000). High water content can cause microbial growth because water is a medium for microbial growth that can damage the compounds contained in the sample (Jubaidah, *et.al* 2022; Wijaya, *et.al.* 2020). The water content requirement according to SNI 01-3729-1995 regarding the quality requirements of sago starch states that the water content contained in the starch is a maximum of 13%. Sago starch contains an amylose content of 28.84% and an amylopectin content of 71.16% (Jading, 2011). The conditions for employing sago starch are the same as for lai fruit seed starch. Lots of amylopectins. Determination of the water content aims to determine the content or amount of water in the extract (Wijaya*, et.al*. 2022). The determination of the water content obtained from the starch of lai fruit seeds was 11.4%. Temperature also has an effect on water content; the higher the drying temperature, the lower the water content (Jubaidah, *et.al*. 2019). Based on these results, it shows that the water content of the lai fruit seed starch has met the specified requirements. This is supported by research conducted by Soebagio & Sriwododo (2009), who stated that the water content of durian seed starch was 10.35%, which also met the requirements because it was still in the same genus.

The total ash content test aims to determine the material's internal and external mineral content (Depkes RI, 2000). The types of minerals contained in a material consisting of organic salts (salts of malic acid, oxalate, acetate, pectate, and others) and inorganic salts (phosphate, carbonate, chloride, sulphate nitrate, alkali metal) (Paramita, *et.al*. 2019). The requirements for ash content according to SNI 01-3729-1995 regarding quality requirements for sago starch state that the ash content contained in starch is a maximum of 0.5%. The determination of the ash content obtained from the starch of lai fruit seeds was 1.5%. These results indicate that the starch ash content of other fruit seeds does not meet the specified requirements. In the Antarlina study (2016), the study's results stated that the ash content of lai fruit was 1.69%, which had approximately the same yield as the starch of lai fruit seeds.

The acid-insoluble ash content test aims to determine the external mineral content in the material (Depkes RI, 2000), which is related to the cleanliness and purity of the sample (Paramita, *et.al*. 2019). In the acid insoluble ash content test, the ash content results were dissolved with dilute HCl so that the mineral content was soluble in acid and that the acid-insoluble ones showed impurities (Rivai*, et.al.* 2013). The value of high acid insoluble ash content or does not meet the requirements can mean the presence of impurities or silicate contamination in samples obtained from the environment around plants, such as sand and heavy metals such as Pb and Hg (Paramita, *et.al.* 2019). The requirement for acid-insoluble ash content according to the Ministry of Health of the Republic of Indonesia in 1979 on seeds states that the acid-insoluble ash content is a maximum of 0.75-1.3%. The results of the research showed that the acid insoluble ash content obtained from the starch of the lai fruit seeds was 0.75%. These results indicate that the acid insoluble ash content in the starch of lai fruit seeds has met the specified requirements.

***Test Results for the Chemical Content of Lai Fruit Seed Starch***

Before the chemical content test, the test solution was prepared—preparation of the test solution by dissolving the starch powder with aqua dest and then adding HCl. The addition of HCl serves to break down the polysaccharides in starch into their constituent monosaccharides (Choirunnisa, 2015).

***Carbohydrate Test***

Molisch test aims to determine the presence of carbohydrates in the sample. The Molisch test can be used to analyze all carbohydrates, including monosaccharides, disaccharides, and polysaccharides (Atma, 2018). The results of examining lai fruit seed starch with the Molisch test can be seen in Figure 2.

**Figure 2.** Yield of lai fruit seed starch by Molisch test

The sample examination results, in this case, the lai fruit seed starch, showed positive results with the formation of a red, purple color resembling a ring so that it can be said that the lai fruit seed starch contains carbohydrates.

***Polysaccharide Test***

Iodine reagent aims to prove the presence of polysaccharides such as starch, glycogen, and cellulose. Starch/starch produces a blue color indicating amylose or purple indicating amylopectin, glycogen produces a red-brown color, and cellulose produces a negative color (Poedjiadi, 2012). The sample examination results with iodine reagent can be seen in Figure 3.

A :starch before hydrolysis     B :starch after hydrolysis

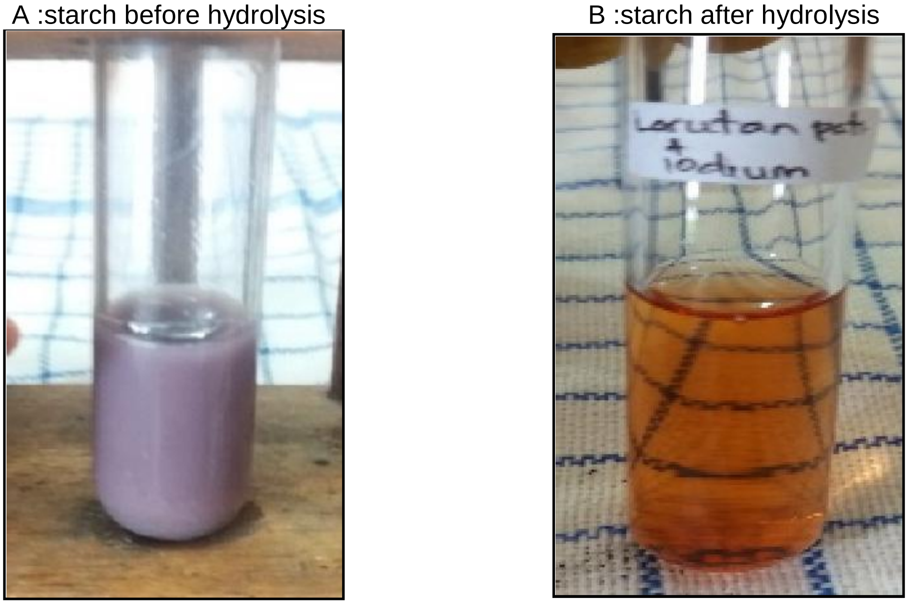

**Figure 3.** Results of starch test of lai fruit seeds with iodine reagent

The test of lai fruit seed starch that has not been hydrolyzed with iodine reagent yields a purple color, indicating that the lai fruit seed starch is positive for starch or starch with higher amylopectin. The starch of other fruit seeds that had been hydrolyzed with HCl did not generate a colour that was still red when mixed with iodine in the experiment, indicating that the starch of other fruit seeds had a negative result. If the starch added with HCl produces negative findings when tested with iodine, it can be assumed that the starch has been thoroughly hydrolyzed (Choirunnisa, 2015).

***Reducing Sugar Test Results***

Fehling's reagent aims to determine the presence or absence of reducing sugars. The sample examination results with Fehling's reagent can be seen in Figure 4.

A : Sample comparison (Glucose)        B :Test sample

**Figure 4.** Sample test results with Fehling's reagent

If Fehling's reagent is added with reducing carbohydrates, then heated, the colour changes from blue-green to yellow-reddish. Finally, a red brick precipitate of cuprous oxide is formed when reducing carbohydrates are largely reduced.

Fehling's reagent is usually used to reduce sugar, which produces a brick-red residue. Still, this test is not specific for aldehydes because the test is optimistic about the formation of green color. These results are supported by Wesly (2015), which states that samples of corn cobs that have been hydrolyzed and tested with Fehling's reagent produce a green color, indicating the presence of reducing sugar, namely fructose. In this case, the results of the sample test showed that the starch of lai fruit seeds produced a green color, so it can be said that the seeds of lai fruit were positive for reducing sugars.

***Thin Layer Chromatography Profile***

The TLC profile aims to determine the components of compounds contained in the sample (Juwita, *et.al*. 2013). The stationary phase used was a silica gel GF254 plate (Wibisono, 2014); before using the plate, the activation was first aimed at removing moisture from the water in the plate (Wulandari, 2003). The polar nature of the silica gel plate and the carbohydrate (sample) and the reference solution (sugar) used creates a polar solution, causing carbohydrates and the reference solution (sugar) to be strongly bound to the stationary phase, so that the mobile step used must be highly polar, such as n-butanol, acid acetate, or aqua dest with a ratio of 12:6:6 (Wibisono, 2014). In this test, a comparison solution was used to determine what sugar content was contained in the starch of other fruit seeds. The results of this TLC method are obtained by comparing the Rf value of the sample with the Rf value of the comparison. The results of the TLC profile examination can be seen in table 5.

**Table 5.** TLC analysis on other fruit seed starch

| Spots | Spot Elution Distance (cm) | Rf |
|---|---|---|
| S:Lai fruit seed starch sample 15% | 7.5 | 0.93 |
| A :Glucose 10% | 7.6 | 0.95 |
| B: Fructose 10% | 7.5 | 0.93 |
| C: Sucrose 10% | 7.55 | 0.94 |
| D: Lactose 10% | 7.4 | 0.92 |

Note: Stationary phase by GF254 silica gel plate; Mobile phase by n.butanol, acetic acid, aquades (12:6:6)

**Figure 5.** Lai Fruit Seed Starch TLC Profile

The Rf value ranges from 0 to 1, and the best Rf value is between 0.2 and 0.8. Based on the TLC profile examination results, it was found that the seed starch of lai fruit produced an Rf value of 0.93. The Rf value obtained in the study did not fall into the category of the best Rf value; this could be because the analyte stain was disturbed by impurities present on the plate. Dirt in the plate is hydrophilic, so the use of a polar mobile phase will cause plate impurities to tend to move to the mobile phase and have a high Rf value (> 0.8) (33). Based on the Rf value of the sample obtained, it is the same as one of the comparison Rf values used, so it can be concluded that the starch of other fruit seeds contains fructose.

## Conclusion

Based on the results of the research above, it can be concluded that the seed starch of lai fruit has the potential to be used as an alternative to new excipients in pharmaceutical preparations.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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