ABSTRACT

Skin aging can occur if endogenous antioxidants are not sufficient to ward off radical compounds and external antioxidant intake is needed. One plant that contains antioxidants is lotus root (Nelumbo nucifera Gaertn.) and has the potential to be developed into nutraceutical products, namely edible film strips. This research aims to develop edible film strips from ethanol extract of lotus roots as anti-aging by optimizing polymer and plasticizer components, namely HPMC and sorbitol using the Simplex Lattice Design method from Design Expert 13.0 software. The antioxidant activity of the preparation was tested using the 1,1-diphenyl-2-picrylhydrazyl method. Phytochemical screening results showed that the extract contained polyphenols, tannins, flavonoids and saponins. The predicted optimal formula for edible lotus root extract has a HPMC-Sorbitol composition ratio of 2:10 with a desirability value of 0.737. Observation results show that the optimum formula preparation has a thickness of 0.32 mm; pH 3.22; drying shrinkage 6.91%; absorption capacity 49.73%; and disintegration time 31.56 seconds. This value is not significantly different from the physical characteristic value predicted by the software (Sig. 2-tailed > 0.05). The potential anti-aging activity which is identical to the antioxidant activity of the optimum formula is shown by obtaining an IC50 value of 58.6174 ppm which is included in the strong antioxidant group. This is thought to be attributed to the presence of tannin and flavonoid polyphenol compounds in the lotus root extract, where these compounds have hydroxy groups which have been proven to be able to capture free radicals.

Keyword: Lotus root, nutraceuticals, edible film strips, optimum formula, antioxidant activity

ABSTRAK

Penuaan kulit bisa terjadi apabila antioksidan endogen tidak cukup menangkal senyawa radikal dan dibutuhkan asupan antioksidan dari luar. Salah satu tanaman yang mengandung antioksidan adalah akar seroja (Nelumbo nucifera Gaertn.) dan berpotensi untuk dikembangkan menjadi produk nutrasetikal, yaitu edible film strips. Penelitian ini bertujuan mengembangkan sediaan edible film strips ekstrak etanol akar seroja sebagai anti-aging dengan mengoptimasi komponen polimer dan plasticizer, yaitu HPMC dan sorbitol menggunakan metode Simplex Lattice Design dari software Design Expert 13.0. Aktivitas antioksidan sediaan diuji menggunakan metode 1,1-difenil-2-pikrilhidrazil. Hasil skrining fitokimia menunjukkan ekstrak mengandung polifenol, tanin, flavonoid dan saponin. Prediksi formula optimum edible ekstrak akar seroja memiliki perbandingan komposisi HPMC-Sorbitol 2:10 dengan nilai desirability 0,737. Hasil observasi menunjukkan sediaan formula optimum memiliki ketebalan 0,32 mm; pH 3,22; susut pengeringan 6,91%; daya serap 49,73%; dan waktu hancur 31,56 detik. Nilai tersebut tidak berbeda signifikan dengan nilai karakteristik fisik hasil prediksi software (Sig. 2-tailed > 0.05). Potensi aktivitas anti-aging yang identik dengan aktivitas antioksidan dari formula optimum ditunjukkan dengan diperolehnya nilai IC50 sebesar 58,6174 ppm yang termasuk dalam kelompok antioksidan kuat. Hal ini diduga karena adanya senyawa polifenol tanin dan flavonoid dalam ekstrak akar seroja, dimana senyawa-senyawa tersebut memiliki gugus hidroksi yang terbukti mampu menangkap radikal bebas.

Kata Kunci: Akar seroja, nutrasetikal, edible film strips, formula optimum, aktivitas antioksidan

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Introduction:

Skin aging is a degenerative process with a tremendous impact on appearance and quality of life, which is believed to be an emerging health issue in accordance with the growing aging population. Lipids play a vital role in skin as a barrier and as building blocks for cell membrane and a variety of bioactive molecules. The latest evidence has indicated that the supplementation of certain types of fatty acids and sterols may modulate skin cell function differential (Wang and Wu 2019). Skin aging is characterized by the thinning of subcutaneous fat tissue, including facial fat, which will cause the appearance of sunken and deep cheeks and the appearance of eye bags (Ahmad and Damayanti 2018).

Skin aging can generally be divided into two categories, including intrinsic aging or chronological aging which is related to aging and extrinsic aging which is related to exposure to external factors. The main external factor that accelerates the skin aging process is exposure to sunlight which contains ultraviolet rays. Indonesia is a tropical country with exposure to ultraviolet rays all year round, so the population of Indonesia is very vulnerable to aging skin, especially extrinsic skin aging due to long-term exposure to ultraviolet light (Ahmad and Damayanti 2018). Disruption of intrinsic factors can cause an increase in free radicals and shortening of telomeres, which further weakens collagen production, while external factors cause abnormal elastin growth (Dewiastuti and Hasanah 2016).

If the endogenous antioxidant system (in the body) is insufficient, external antioxidants such as polyphenolic compounds are really needed (Andarina and Djauhari 2017). One example of a plant that is rich in antioxidants is lotus root (Nelumbo nucifera Gaertn.). There is research showing that lotus roots contain flavonoids and other polyphenolic compounds such as gallic acid, catechin, epicatechin, and gallocatechin (Yi et al. 2016). In other research, it has been shown that ethanol extract of lotus root can inhibit skin damage by reducing the level of metalloproteinase type MMP-1 which is known to degrade collagen (Iwamoto et al. 2022; Murlistyarini and Dani 2022).

The polyphenol content in lotus roots has the potential to be developed into nutraceuticals rich in antioxidants that are needed by society. Nutraceuticals are substances that are or are part of food that provide medicinal or health benefits, including the prevention and treatment of disease (Nasri et al. 2014). There are many different forms of nutraceuticals, one of which is edible film strips. Edible film strips are thin oral films that can be easily placed on the tissue of the tongue or oral mucosa, which are immediately wetted by saliva and then quickly dissolved and released. Edible film strips have unique characteristics, which have good mucoadhesive properties, dissolve easily and quickly release active ingredients. When viewed from an economic perspective, edible film strips are lighter, easier to carry, and easier to consume compared to tablets or other oral preparations (Wahyuni, Rikmasari, and Maulidiah 2021; Saputri et al. 2021).

Based on the description above, research is needed to develop edible film nutraceutical preparations from ethanol extract of lotus roots as an antioxidant to prevent and treat skin damage due to aging. Edible film preparations have unique characteristics, namely the form of a thin film, does not stick to the tongue, has good mucoadhesive power, dissolves quickly and can quickly release substances. Edible film can be produced from materials that have the ability to form film or film-forming ability. In the manufacturing process, the film-making material must be dissolved and dispersed in a solvent such as water (Ismaya, Fithriyah, and Hendrawati 2021). Excipient components that have a large influence on the characteristics of edible films are polymer excipients and plasticizers. One of the excipients that has this function is Hydroxy Propyl Methyl Cellulose (HPMC) and Sorbitol. Based on this background, research was carried out to obtain the composition of HPMC and edible sorbitol from lotus root extract that was in accordance with the optimum formulation, and characterization was carried out so that the physical properties could be determined and potential anti-aging activity tested, which was
identical to the antioxidant activity of the optimum formula.

Methods:
Preparation of Simplicia and Extraction of Seroja Roots

Preparation of lotus root simplicia is done by wet sorting fresh lotus roots and slicing them thinly with a thickness of ± 1 mm, then drying the slices at a temperature of 70°C. The resulting dried simplicia is crushed and sifted. The simplicia powder was then extracted using 70% ethanol for 5 days (Iwamoto et al. 2022). The ethanol extract of the lotus root was then evaporated using a rotary evaporator at a temperature of 50°C until a thick extract was obtained. On the thick extract of lotus root, phytochemical screening was then carried out for the content of polyphenols, flavonoids, alkaloids, saponins and tannins. The positive results of the compounds in the extract obtained were reconfirmed using the Thin Layer Chromatography (TLC) method.

Formulation and Optimization of Edible Film Strips

The components in the formula that were optimized were HPMC as a polymer with a concentration range of 2-6% and sorbitol as a plasticizer with a concentration range of 6-10%. The comparison of the two components is determined using the Simplex Lattice Design (SLD) method. The expected response of the physical characteristics of the preparation is thickness, pH, drying loss, dissolution time and water absorption of the edible film. From this system, 8 run formulas were produced with the ratio of sorbitol and HPMC as shown in Table 1. Next, edible film strips were prepared according to the formula and the respective Sorbitol-HPMC ratios produced.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Run</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium Saccharin</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Menthol</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Aquadest</td>
<td></td>
<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
</tr>
</tbody>
</table>

The edible film mass mixture was printed on a glass plate measuring 20x20x0.5 cm and then dried using a drying cabinet. Each preparation produced was cut into 2x3 cm pieces and tested for physical characteristics (Wahyuni, Rikmasari, and Maulidiah 2021). The physical characteristics test results were then analyzed using response surface methodology with the help of Design Expert 13.0 software.

Physical Characteristics Test of Edible Film Strips

The resulting edible film strips of lotus root extract were tested for the physical characteristics of the preparation, including thickness, pH, drying shrinkage, dissolution time, and water absorption test. The thickness of the preparation is determined based on the average results of measurements at 5 different points. The pH of the preparation is measured using a calibrated pH meter and a good preparation has a pH that matches the pH of the mouth, namely between 5.5-7.9 (Harmely, Deviarny, and Yenni 2014). The drying shrinkage percentage was determined using a constant porcelain cup. Next, a number of edible film preparations are heated in an oven at 105°C for 2-5 hours until the weight remains constant (Wahyuni, Rikmasari, and Maulidiah 2021).

The dissolution time of edible film was determined by observing the time required for the film to completely dissolve after being placed in a petri dish containing 2 mL of distilled water. A good edible film preparation has a disintegration time of between 5-30 seconds (Wahyuni, Rikmasari, and Maulidiah 2021). The water absorption capacity of edible film is determined by placing the weighed preparation in a container containing distilled water for 10 seconds. Next, the preparation was dried from the adhering water and weighed. The treatment was repeated until the difference in the final weight of

Table 1. Composition of Optimization Components

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Run</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td></td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>HPMC</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Sorbitol</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>PVA</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
the preparation was no more than 0.2 gram (Syiami, Handayani, and Najihudin 2021).

Anti-Aging Activity Test of Edible Film Strips Seroja Root Extract in Vitro

One effort to overcome aging can be determined by testing the antioxidant activity of the Edible Film Strips Seroja Root Extract preparation using the 1,1-diphenyl-2-picylhydrazyl (DPPH) method. A number of edible film samples were dissolved in 96% ethanol to obtain a stock solution with a concentration of 500 ppm. Next, it is diluted to 50-90 ppm in a 25 mL volumetric flask, added with DPPH solution and methanol p.a. up to the limit mark. Measurement of the absorbance of the test solution was carried out after incubation for 30 minutes in light-protected conditions (dark) and the absorbance was measured at the maximum wavelength. (Tutik et al. 2021).

Results:

In this research, the part of the plant used was fresh lotus root. The process begins by cutting the lotus roots as thinly as possible to speed up the drying process. The drying process is carried out in a drying cabinet without being exposed to direct sunlight. Dried simplicia lotus roots are ground to reduce their size into simplicia powder. The process of extracting compounds from lotus roots is carried out using the maceration method. The filtrate obtained was then concentrated using a rotary vacuum evaporator and then thickened using a water bath until a thick extract was obtained. The drying process to obtain a thick extract is shown in Figure 1.

Figure 1 shows lotus roots were sorted and dried to obtain 499.5 grams of dried simplicia which was then macerated and an extract yield of 19.51% was obtained. The thick extract was screened for phytochemicals with color reagents and confirmed using Thin Layer Chromatography (TLC) as shown in Table 2.

Table 2. Phytochemical Screening Results of Seroja Root Extract

<table>
<thead>
<tr>
<th>Compound</th>
<th>Color Reaction</th>
<th>TLC</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols</td>
<td>FeCl₃</td>
<td>Green black</td>
<td>Vanilin-HCl</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Mg powder + HCl</td>
<td>Amyl alcohol layer is orange</td>
<td>Ammonia vapor</td>
</tr>
<tr>
<td>Tannin</td>
<td>FeCl₃</td>
<td>Blue black</td>
<td>FeCl₃</td>
</tr>
<tr>
<td>Saponins</td>
<td>Hot water + HCl</td>
<td>Stable foam</td>
<td>Anisaldehyde-id-H₂SO₄</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer</td>
<td>No precipitate is formed</td>
<td>-</td>
</tr>
</tbody>
</table>

Preliminary tests of the ethanol extract of lotus roots were carried out by color reaction and confirmation of the content of polyphenolic compounds, flavonoids, tannins, saponins, and alkaloids using TLC. Based on the results of the color reaction test and TLC, it was shown that the ethanol extract of lotus roots contains polyphenols, flavonoids, tannins, and saponins.

The effect of the Sorbitol-HPMC component on the response tested based on the analysis results from Software Design Expert 13.0 is listed in Figure 2.
An edible film strip antioxidant activity test of lotus root ethanol extract preparations made with the optimum Sorbitol-HPMC component formula was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. In this test, edible film strips were prepared as test samples, and vitamin C was compared at a maximum wavelength of 515.8 nm. The test results were interpreted using the IC50 parameter which was determined using a linear regression equation from the relationship curve between sample concentration and % inhibition and produced IC50 data as listed in Table 3.

<table>
<thead>
<tr>
<th>Replication</th>
<th>IC50 Value (ppm)</th>
<th>Average (ppm)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>56.8533</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>63.0748</td>
<td>58.6174</td>
<td>3.89</td>
</tr>
<tr>
<td>III</td>
<td>55.9241</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion:
Lotus Root Extraction
The part of the lotus plant used is the fresh root part. The drying process is carried out in a drying cabinet for 24 hours. The sample drying process aims to stop enzymatic reactions and
Testing of the pH of the edible strips preparation of lotus root extract was carried out to determine the suitability of the pH of the preparation with the range of oral pH requirements. This is to ensure safety and comfort when the preparation is used, where a good edible preparation is one that is in accordance with the pH of the mouth. The edible lotus root extract strip preparation tends to have an acidic pH, namely 2.81 to 3.38. This is not in accordance with previous research indicating that edible film strip preparations are suitable for oral pH, namely 4.5 to 9, and preferably around 6.5 to 7.5 (Issusilaningtyas et al. 2019). Based on graphic image 2(b), it can be seen that increasing and decreasing the concentration of HPMC and sorbitol used can reduce the pH of the edible preparation. Apart from that, the acidity of this preparation can be caused by the use of citric acid in the edible formula. Oral preparations that are too acidic can cause irritation to the oral mucosa and affect the hydration level of the polymer used, namely HPMC (Chen, Bunt, and Wen 2015).

Determination of drying shrinkage of edible strips preparations is identical to the water content lost from the preparations after the heating process. Loss of water can affect the shape and texture of the preparation. This can be a consideration in determining how to store the preparation and to maintain the water content of the preparation, so that the texture and shape of the edible strips will be maintained during storage (Wahyuni, Rikmasari, and Maulidiah 2021). Based on the graphic image 2(c), it can be seen that increasing the concentration of HPMC and sorbitol used tends to increase drying losses which also indirectly indicates a higher water content. The high water content in the preparation is actually undesirable because water is a growth medium for microorganisms. Each preparation formula for edible film strips of lotus root extract meets the requirements, because the drying loss of tial is more than 9.29% (Tyas, Meinitasari, and Septianingrum 2018).

The dissolution time of an edible strip preparation is the time required for the preparation to completely dissolve after contact with the saliva solution. The requirement for a
good dissolution time for edible preparations is between 5-30 seconds (Tanjung, Julianti, and Rizkiyani 2021). Based on graphic image 2(d), it shows that increasing the concentration of HPMC and sorbitol will increase the dissolution time of the preparation. This is because HPMC is a cellulose derivative polymer which has a high viscosity and causes water diffusion into the preparation to be low and the preparation will take longer to dissolve.

Determination of the absorption capacity of edible preparations to water is expressed in percent swelling (%swelling), where the greater the percentage produced indicates the greater the absorption capacity of the preparations to water. Based on graphic image 2(e), it shows that the higher the concentration of HPMC and sorbitol, the more the absorption capacity of the edible preparation increases. The great ability of edible strips to absorb water is more due to the presence of the HPMC component which is a cellulose derivative which has OH groups, where these groups easily bind with water (Ghadermazi et al. 2019).

Based on the results of the optimum formula analysis with the optimization parameters used, namely thickness, pH, drying loss, dissolution time and absorption capacity, the optimum formula for edible film strips from lotus root extract was produced with a composition of 2% HPMC and 10% Sorbitol with a desirability value of 0.737 as shown in Figure 2(f). In determining the optimum formula, the desired goal value for each response has a minimum value. It is desirable to prepare edible film strips with an optimal formula to produce edible preparations that have the thinnest possible thickness, with an appropriate pH, low drying loss, dissolve quickly and have low water absorption capacity.

**Optimum Formulation Verification**

Verification of the optimum formula for edible film strips for lotus root extract is necessary to check the suitability between the predicted formula from the Design Expert software and the actual observed formula and to ensure that the predicted optimum formula can produce the same preparation in each manufacture. Based on the verification results of the optimum formula, the significance value was >0.05, so that all physical characteristic values observed were not significantly different from the predicted value of the optimum formula for edible strips of lotus root extract. These results show that the equations produced by the Design Expert 13.0 software can be used to predict the thickness, pH, drying loss, absorption capacity and dissolution time of the lotus root extract edible strips preparation using a combination of HPMC polymer and Sorbitol plasticizer.

The observation results obtained were compared with predicted data from Design Expert software using One Sample T-Test with SPSS version 23 with a confidence level of 95%. The T test can be carried out on data that is normally and homogeneously distributed. Based on Table 2, the resulting significance value (2-tailed) is >0.05 and shows that there is no significant difference between the physical characteristics of the observation results and the results predicted by the Design Expert software.

**Test of the Antioxidant Activity of Edible Film Strips Seroja Root Extract**

The DPPH method is used to test the antioxidant activity of edible film strips of lotus root extract because it is easy, simple and has a high sensitivity, and is capable of analyzing large quantities of samples in a relatively short time (Setyowati and Damayanti 2018). DPPH free radicals is the fastest and easiest method compared to the FRAP and FIC methods. Testing using this method requires an incubation process because the DPPH solution is easily degraded and is very sensitive to light (Wahidah 2020). The value of antioxidant activity is indicated by the IC50 value, which is the concentration value of antioxidant compounds needed to inhibit DPPH free radical activity by 50%. The smaller the IC50 value, the greater the antioxidant activity in warding off free radicals (Tukiran et al. 2019).

The IC50 value results is presented in Table 3 which shows that the edible film strips preparation of lotus root extract has an antioxidant activity of 58,6174 ppm. This IC50 value is classified as strong antioxidant activity because the IC50 value is between 50-100 ppm (Koto et al. 2019). This is thought to be due to the...
presence of polyphenolic compounds such as tannins and flavonoids contained in lotus root extract, where these compounds have been proven to have antioxidant activity originating from the OH group in the structure of the compound. Compounds with OH groups attached directly to aromatic hydrocarbon ring groups are called phenolic compounds. The hydroxyl group functions as a donor of hydrogen atoms when reacting with radical compounds through an electron transfer mechanism, so that the oxidation process can be inhibited (Sukma, Nurlansi, and Nasrudin 2022). Antioxidant activity is also due to the saponin content in lotus root extract, where saponin compounds are included in glycoside compounds which have been proven to be able to reduce free radical compounds (Putri et al. 2023). Lotus root extract, which contains antioxidant compounds, is able to protect the fat oxidation process, thus protecting the skin, especially the stratum corneum layer, from attacks by fatty peroxyls (Nur, Rumiyati, and Lukitaningsih 2017).

Conclusions:

The optimum formula for edible film strips from lotus root extract has a HPMC-Sorbitol composition ratio of 2:10. The preparation has a thickness of 0.32 mm; pH 3.22; drying shrinkage 6.91%; absorption capacity 49.73%; disintegration time 31.56 seconds; and the IC₅₀ value is 58.6174 ppm, where this value is included in the strong antioxidant group.

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