The Potency of Liquorice Extract (Glycyrrhiza glabra L.) as Skin Whitening

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Abstract

Liquorice (Glycyrrhiza glabra L.) contains flavonoids such as glabrene, isoliquiritigenin and glabridin which have a functions as a tyrosinase inhibitors in the process of melanogenesis in the melanocytes so that potentially as a skin Whitening. The aim of this research is to find the quality of extracts of standardized liquorice extract and potential as a skin whitening. The extraction of liquorice powder was performed by kinetic maceration with ethanol 96% and concentrated on a rotary vacuum evaporator at 175 mmHg, 60 rpm, and 40 °C. Phytochemical examinations conducted by Farnsworth method, examination of quality parameters of extract, which include specific parameters and non-specific parameters, microbial contaminant, and the total flavonoid content. The research results that liquorice extract contain flavonoids compounds, a thick extract, dark brown, aromatic odour, sweet taste with fulfilled specific and non-specific parameter such as the compound contents dissolved in water and ethanol, loss on drying, moisture content, total ash content, ash content acid insoluble, residual solvents were 70.73%; 75.37%; 6.03%; 5.15%; 4.53%; 0.98%; 0.43%. Heavy metal (Cd and Pb) contaminant were 2.73% and 0.05%. Total plate Count & Yeast & Mold Count Plate were 5.00 x 10 colony/g and 5.33 x 10 colony/g. Total flavonoid content was 1.31%. It can be concluded that the liquorice ethanolic extract is fulfilled the extract quality requirements and potentially as a skin whitening.

Keywords: Glycyrrhiza glabra L, Skin Whitening, Extract

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Introduction

Beautiful skin can be consider as a white skin using whitening cosmetics which is causing trends and becomes skin lightening demand. Generally, many outstanding whitening cosmetics contain harmful chemicals such as mercury and hydroquinone. Whitening cosmetic allowed should be safe, no side effects but still potent. They could be derived from natural ingredients such as liquorice (Glycyrrhiza glabra L.).

Liquorice plant contains chemicals such as glycyrrhizin, saponins, glycosides isoliquiritigenin, asparagine, umbeliferona, glabren, glucose, sucrose, acid likuiritat, hydroksiglsirhitat acid, essential oils, asparagines, main secondary metabolites from the flavonoid e.g. glabridin that serves as tyrosinase inhibitors (Yokota, et. Al., 2008).

Tyrosinase inhibitors associated with melanin pigment produced by melanocyte cells consisting of pigment eumelanin which shares brown-black colour and feomelanin shares yellow-red colour. In normal circumstances, the production of melanin pigment is stable, but in certain circumstances the production of melanin changes, for example sun exposure, hormonal changes, effects of smoking and alcohol. The process of formation of melanin in the human body can be reduced by several mechanisms, such as antioxidants, enzyme tyrosinase inhibitors and hormonal activity. The process of the formation of melanin or pigment in human skin occurs in the presence of biocatalyst (enzyme) and ultraviolet light. Biocatalyst that plays an important role in the production eumelanin is tyrosinase found in animals, plants and humans. The glabridin in licorice extract serves as a tyrosinase inhibitor, then the process of the formation of melanin pigment in the epidermis layer of the skin can be inhibited without affecting the DNA thus the skin looks whiter and brighter (Draelos ZD 2006; Chang TS, 2000).

This research used the liquorice which is non-cytotoxic and effective as a natural tyrosinase inhibitor (G. Diaz 2009). This study aimed to obtain a standardized extract of liquorice and to determine the total flavonoid that can indicate adanta glaridin compounds which have potential as a skin whitener. Standardization in the pharmacy is a series of parameters and measurement procedures and the results which are related to elements of the paradigm of pharmaceutical quality, in terms of qualified standard (chemical, biological, and pharmaceutical), including guarantees (limits) as the stability of...
pharmaceutical products. Quality requirements of extracts consist of various parameters of the general and specific parameters. Fulfilment of quality standards of products/materials cannot be separated from the process control. It means that the process is also standardized to ensure the production of standardized product (Dep.Kes, RI, 200).

Liquorice extract prepared by maceration kinetics using 96% ethanol which is universal solvent that can withdrawal all secondary metabolites. to get a standardized extract of liquorice, the extract was determined its quality parameters consisting of specific parameters (organoleptic, water and ethanol soluble content) and non-specific parameters (loss on drying, water content by Karl-Fischer titration method, total ash content and acid insoluble ash content, residual solvents by gas chromatography, heavy metal contamination by atomic absorption spectrophotometry and pathogenic microbial contamination) as well as the determination of the total flavonoids using UV-Vis spectrophotometry (Dep. Kes RI 2000; Tian M, 2009).

**Methodology**

**Materials**

Crude liquorice (Glycyrrhiza glabra L.), ethanol 96%, ammonia 10% and 25%, chloroform P, LP Dragendorff reagent, Mayer reagent LP, 1:10 hydrochloric acid, magnesium powder P, concentrated hydrochloric acid, amyl alcohol P, iron (III) chloride (1%) P, 1% gelatine, Stiassny reagent, Nutrient agar (NA), Potato Dextrose Agar (PDA), distilled water.

The tools used are UV-Vis spectrophotometer (UV-1800, Shimadzu), atomic absorption spectrophotometer (AA-6800, Shimadzu), gas chromatography (GC-17 A, Shimadzu), moisture meter (Metrohm), analytical balance, macerator Kinetic, vacuum rotary evaporator, ovens, furnaces, incubators, desiccators, water bath, blender, petri dish, glass tools.

**A. Extraction**

Simplicia powder of liquorice was extracted by maceration kinetic 4 x 24 hours using 96% ethanol thus the active compounds are not decomposed. The extract obtained was concentrated by rotary evaporator and condensed water at a temperature of >50 °C produced by hot steam. Calculate DER which is equal to amount of crude drug which produces one gram of extract.

**B. Determination of Extract Parameter**

1. Specific parameters including organoleptic and water and ethanol content.

<table>
<thead>
<tr>
<th>Simplicia (g)</th>
<th>The amount of extract (g)</th>
<th>DER</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>261.66</td>
<td>3.83</td>
<td>26.13</td>
</tr>
</tbody>
</table>

**2). Non-specific parameter including loss on drying, water content, total ash content and acid insoluble ash content by gravimetric, residual ethanol by gas chromatography, heavy metal contamination using atomic absorption spectroscopy, Total number of CFU using liquid Nutrient Agar and molds and yeasts number of colony using liquid Potato Dextrose Agar that the number of growing colonies was counted by colony counter.**

**C. Determination of Total Flavonoid**

Reagent: Solution hexamethylenetetramine (HMT) 0.5% w/v; HCl 25%; glacial acetic acid 5% v/v in methanol; 2% AlCl₃ solution glacial acetic acid.

The concentrated test solution: The extract was put into the flask and added HMT solution, acetone and HCl then refluxed. The mixture was filtered using cotton; the filtrate was put into the flask. The residue was refluxed again until all the compounds were perfectly extracted.

Acetone and water were added to the filtrate in a separating funnel. The mixture was extracted with ethyl acetate. Ethyl acetate fraction was collected and added to the ethyl acetate. Blank solution: The concentrated test solution in glacial acetic acid.

Test solution: The concentrated test solution in AlCl₃ solution and glacial acetic acid.

Measurements: done 30 minutes after the addition of AlCl₃ using a spectrophotometer at a wavelength of 280 nm.

**Results and Discussion**

**Microscopy Identification of Liquorice**

Microscopic identification is carried out to ensure the identity of the simplicia used in research. The results of the microscopic identification are in accordance with the literature Materia Medika Indonesia II.

**Preparation of Extracts and DER**

1000 g liquorice powder was extracted by kinetic maceration at room temperature using 96% ethanol as much as 25 L. The results of the calculation of DER and yield the following phytochemical screening were shown in Table 1.
Phytochemical screening of powder and 96% ethanol extract of Liquorice were conducted to determine secondary metabolites which became the basis for biological activity. Results of phytochemical screening of the powders and extracts of liquorice displayed that the extract contains flavonoids, saponins, tannins, quinones, and triterpenoid.

**Extract Quality of Specific Parameter**

**Organoleptic**

Organoleptic examination aimed at early recognition of the extract which signifies the hallmark of the extract that is subjective. Organoleptic test results indicated that the extract can be extracted by using ethanol extract of liquorice are thick, blackish brown, aromatic smell with a sweet taste.

**Soluble Compounds**

Compounds in the powder and extract were extracted using 96% ethanol and water-chloroform. The results obtained from the extraction with water showed 20.12% (powder, F1 qualify IV, ≥ 20%) and 70.73% (extract) and with ethanol 26.75% (powder) and 75.37% (extract, F1 qualify IV, ≥ 75%). Levels of soluble compounds showed that many secondary metabolites are present in the solvents.

**Loss on Drying and Water Content**

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Powder (%)</th>
<th>Extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Loss on drying</td>
<td>4.97</td>
<td>6.04</td>
</tr>
<tr>
<td>2.</td>
<td>Water content</td>
<td>4.10</td>
<td>5.15</td>
</tr>
</tbody>
</table>

The results of loss on drying of powders and extracts mean that water evaporates from the extract after heating at a temperature of 105 °C. The results meet the requirements of the Indonesian Pharmacopoeia IV edition stated that the value is not more than 7%. Water content in the powder and extract showed the amount of water that affect the stability of the extract because water is a good medium for growth of microorganisms.

**Total Ash Content**

Determination of total ash content aims to determine the total mineral content, both physiological and non-physiological minerals in the extract. The results of the determination of total ash content and acid insoluble ash content met the requirement.

**Residual Ethanol Content**

The results of examination of residual ethanol content in the extract still meet the requirements of the maximum limit of residual solvent in the extract, which is 1.0%. The spectrum of the gas chromatography test results can be seen in the Figure below.

**Heavy Metals Contamination**

Maximum limit of Heavy metal content in the extract is still fulfil the requirement based on Monograph of Medicinal Plant of Indonesia.

**Microbe Contamination**

The results total number of CFU and mold and yeast number of colony were fulfil the requirement based on Extract Monograph of Medicinal plant of Indonesia.

**Total Flavonoids**

The results of examination of total flavonoids in the extract still meet the requirements of the maximum limit of residual solvent in the extract, which is 1.0%. The results of the gas chromatography test results can be seen in the Figure below.

**Table 4. The Results of Heavy Metals Identification**

<table>
<thead>
<tr>
<th>No.</th>
<th>Heavy metals</th>
<th>Amount (mg/kg)</th>
<th>BPOM requirement (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pb</td>
<td>2.7271</td>
<td>≤ 10</td>
</tr>
<tr>
<td>2.</td>
<td>Cd</td>
<td>0.0484</td>
<td>≤ 0.3</td>
</tr>
</tbody>
</table>

**Table 5. Results of Heavy Metal Identification**

<table>
<thead>
<tr>
<th>No.</th>
<th>Assessment</th>
<th>Number (colony/g)</th>
<th>Requirement (colony/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total</td>
<td>5.00 x 10</td>
<td>≤ 1 x 10⁴</td>
</tr>
<tr>
<td>2.</td>
<td>Mold and Yeast</td>
<td>5.33 x 10</td>
<td>≤ 1 x 10³</td>
</tr>
</tbody>
</table>

The results of examination of total flavonoids in the extract still meet the requirements of the maximum limit of residual solvent in the extract, which is 1.0%. The spectrum of the gas chromatography test results can be seen in the Figure below.
Total flavonoid content as shown in the table 6 derived from various types of flavonoids. One of which is glabridin.

**Conclusion**

Liquorice extract as tyrosinase inhibitors has been proven its potential to reduce the pigment melanin thus gives the effect of a natural whitening/lightening.

**Acknowledgment**

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**References**


Chang TS. An Updated Review of Tyrosinase Inhibitors. Taiwan: Department of Biological Science and Technology, National University of Tainan; 2009. p.2440-2, 2449


