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WATER PHASE CONCENTRATE SOLUBILITY TEST SNAKEHEAD FISH EXTRACT (*Channa Striata*) IN ORGANIC COSOLVENT

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ABSTRACT

Snakehead fish extract has been recognized for its potential in healing postoperative wounds and burns. The extract contains albumin, which aids in tissue formation and healing processes. To create a high-quality wound healing drug, the extract needs to be formulated into a concentrated form and combined with a solvent that can effectively dissolve its active substances. Understanding the solubility profile of the extract and the composition of the solvent is crucial for this purpose. This study aims to determine the solubility profile of the aqueous phase concentrate of snakehead fish extract in an organic co-solvent. Solubility is an important parameter as it can impact the release rate and therapeutic effectiveness of the drug. A solubility test was conducted to assess the solubility of the aqueous phase concentrate in various solvents. The results of the solubility test indicated that the aqueous phase concentrate of snakehead fish extract exhibited good solubility in aquadest (20%), slight solubility in propylene glycol (8.5%) and honey (2%), and was insoluble in ethanol, glycerin, mannitol, and sorbitol (0%). These findings provide valuable information for formulating the extract into a wound healing drug with an appropriate solvent composition, ensuring optimal solubility and therapeutic efficacy according to pharmacopeial standards.

Keywords: Concentrate; Aqueous phase of snakehead fish extract; Solubility; Solvent; Polarity Properties

INTRODUCTION

Indonesia, being a maritime country, boasts abundant natural water resources including various fish and marine products. The Indonesian people have long utilized these resources as a source of food and even for medicinal purposes. One such fish with medicinal potential is the snakehead fish (Channa striata). In line with advancements in medical technology, the extract derived from snakehead fish is now being utilized as a postoperative wound healer. Apart from its wound-healing properties, snakehead fish extract contains albumin, which can aid in preventing pulmonary and kidney swelling as well as blood clotting. Serum albumin, a vital component derived from human blood, is typically required for wound healing. However, obtaining serum albumin can be costly for patients. By harnessing the albumin found in snakehead fish, the expenses associated with acquiring serum albumin can be significantly reduced. This is because albumin constitutes the majority of proteins in plasma, accounting for approximately 60% (Safruddin et al., 2019).

The lack of knowledge in processing snakehead fish can have a negative impact on the quality and effectiveness of the fish. Furthermore, the characteristic fishy taste and unpleasant odor of snakehead fish make it unappealing to some individuals. However, considering its beneficial properties, snakehead fish can be utilized as a wound healing drug.

To develop drug preparations, it is essential to understand the solubility profile of the aqueous phase concentrate of snakehead fish extract in organic co-solvents. The solubility of a substance can be influenced by various factors, including the addition of specific solvents, which in turn can be influenced by the solvent's polarity (Alishlah, Wisudyaningsih and Ameliana, 2014).

Polar solvents dissolve polar and ionic substances, and vice versa. The solubility of a substance also depends on its molecular structure, including the ratio of polar and nonpolar groups. Sometimes, a substance is more soluble in a mixed solvent than in a single solvent, which is known as cosolvency. In such cases, the co-solvent, which increases the solubility of the substance, plays a crucial role. Ethanol, glycerin, and propylene glycol are examples of co-solvents commonly used in the pharmaceutical industry, particularly in the production of elixirs.

The solubility of a drug is a crucial parameter as it can impact both the therapeutic effect of the drug and its release rate. Poor solubility of active substances can affect the drug's concentration in the preparation and compromise its quality (Prisukarno et al., 2018) Proteins, in particular, are known to be unstable in most polar solvents like ethanol, highly stable in non-polar solvents like cyclohexane, and even more stable under vacuum conditions. Previous studies have demonstrated that protein solubility is significantly lower in polar solvents such as ethanol and that proteins are essentially insoluble in non-polar solvents like cyclohexane. Therefore, conducting solubility tests using various solvents and co-solvents is essential to develop drug preparations of high quality and efficacy.

METHODOLOGY

Materials

The materials used in this study included snakehead fish (Channa striata), aquadest, ethanol, glycerin, propylene glycol, mannitol, and kelulut honey.

Methods

1. **Sampling**

   The snakehead fish (Channa striata) used in this study was obtained through fishing in Parit Toampe, RT. 013/RW.005 Dusun Cempaka, Sungai Itik Village, Sungai Kakap District, Kubu Raya, West Kalimantan.

2. **Determination**

   The determination of snakehead fish (Channa striata) was conducted in the Biology laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak, West Kalimantan.

3. **Sample Processing**

   The meat part of the snakehead fish was used for the study, while the bones, skin, scales, entrails, and head of the snakehead fish were not utilized. A total of 3 kg of cleaned snakehead fish without the head, entrails, and scales was weighed. The snakehead fish meat was steamed in a pan at a temperature of 60-70°C for 30 minutes. After steaming, the meat was wrapped in a cotton cloth and placed into a hydraulic press. The snakehead fish extract obtained from the press was transferred into a test tube, covered with plastic wrap, and subjected to centrifugation at 6000 rpm for 60 minutes.

   The top layer, which contained oil/fat, was separated from the water phase of the snakehead fish extract. The aqueous phase of the extract was stored in a container and covered with plastic wrap and aluminum foil. The water phase was then freeze-dried, a process conducted at the Engineering Laboratory of Pontianak State Polytechnic. The resulting dried snakehead fish extract was blended until it formed a concentrated powder. The powder was sieved using a mesh with a number 40 sieve to ensure a uniform consistency. Finally, the concentrated powder of snakehead fish extract was transferred to a tightly sealed, dark glass container and stored at room temperature.

4. Protein Level Test

The protein concentration test of the water phase concentrate of snakehead fish (Channa striata) extract was conducted at the Integrated Research and Testing Laboratory of Gadjah Mada University.

5. Solubility Test

The solubility test was conducted using a fast stirring technique with a magnetic stirrer at a temperature of 20°C (Indonesia, 1979). One gram of snakehead fish extract concentrate was gradually dissolved into various organic solvents or co-solvents. The maximum amount of concentrate dissolved in each solvent was recorded. A statistical test was then performed, comparing the solubility results of each material using a bar chart.

RESULT AND DISCUSSION

1. Sampling

The study used the concentrate of the water phase of snakehead fish extract. The snakehead fish (cork fish) used in the study were sourced from fish anglers operating in the Cempaka Hamlet, Sungai Itik Village, Sungai Kakap District, Kubu Raya Regency, West Kalimantan. The criteria for selecting snakehead fish included a length range of 30-50 cm and a weight range of 500-1000 grams per fish. These criteria were based on research conducted by SNI (Suwandi, Nurjanah and Winem, 2014). to ensure the optimal protein content of the snakehead fish.

2. Determination

The purpose of animal determination is to accurately identify and verify the species used in the study to avoid any potential errors in data collection or analysis. The determination of the animal used in this study was conducted at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) at Tanjungpura University (UNTAN). The results of the determination confirmed that the animal used in the study was indeed a snakehead fish (Channa striata) with the assigned identification number 044/A/LB/F.MIPA/UNTAN/2023. This identification ensures the reliability and accuracy of the research findings.

3. Sample Processing

The isolation process of snakehead fish concentrate involves several steps. Firstly, live snakehead fish are sorted and selected based on specific criteria such as intact scales, good body condition, and a bright appearance. Organoleptic criteria, including appearance, meat texture, and odor, are also considered. The selected live snakehead fish are then washed thoroughly with running water to remove impurities. Afterward, the fish is cut into several parts, and the cork fish simplicia (raw material) is packed and stored in the freezer at temperatures between -20°C and -10°C to preserve its quality (Suwandi, Nurjanah and Winem, 2014). The packaging of snakehead fish simplicia follows the guidelines outlined in SNI 2729:2013, ensuring cleanliness, non-contamination of the product, and the use of suitable materials. Packaging is done quickly, carefully, and in a sanitary and hygienic manner (Daisa, Andrie and Taurina, 2017). The snakehead fish meat is wrapped in flannel cloth or cloth napkins and steamed for approximately 30 minutes on a gas stove at a temperature of 70°C. This process helps break down the meat cells and facilitate the optimal extraction of nutrients during the pressing process. The extract is then subjected to repeated pressing to extract the snakehead fish extract. Subsequently, the extract is centrifuged for one hour at a speed of 6000 rpm to separate the aqueous phase from the oil phase, resulting in the water phase of snakehead fish extract.

The results of centrifugation are separated using a syringe or dropper. The aqueous phase and the oil phase of the snakehead fish extract obtained were stored in a container and tightly closed with aluminum foil to prevent contamination. The aqueous phase was filtered using filter paper to separate the dregs from the aqueous phase. The results of the aqueous phase obtained were added with DMDM Hydantoin as a preservative of as much

as 1%. The filtrate is wrapped and put into an ice flask and put in ice cubes so that it is not damaged during shipping to be freeze-dried. The purpose of the freeze dryer is to maintain the simplicia quality of snakehead fish meat so that it is not damaged.

4. Protein Concentrate Test Result

The protein content test conducted on the snakehead fish concentrate revealed a protein level of 99.49%. This indicates that the snakehead fish concentrate is rich in protein compounds, with a very high protein content.

5. Snakehead Fish Concentrate Solubility Test Result Aquadest Solvent

Snakehead fish concentrate exhibits good solubility in water-based solvents. Animal proteins, including those found in snakehead fish, generally have a higher water holding capacity compared to vegetable proteins. This is attributed to the presence of more amine groups in animal proteins. The water absorption of proteins increases with their polarity, especially at pH levels higher than their isoelectric point. Aquadest, also known as pure water or distilled water, is a solvent commonly used in research and pharmaceutical applications. It typically contains very low mineral content. H2O contains almost no minerals (Soderberg and Johanna, 2013). In the solubility test conducted, snakehead fish concentrate showed a solubility percentage of 20% in aquadest solvent, indicating good solubility in water-based solvents.

Albumin is a protein composed of a single polypeptide chain with a molecular weight of approximately 66.4 kDa, consisting of 585 amino acids. This corresponds to the KD value in distilled water, which is close to the KD value of albumin. Distilled water, with the chemical formula H2O, consists of two hydrogen atoms covalently bonded to one oxygen atom. The presence of hydrogen bonds gives distilled water specific properties, such as being a highly effective solvent and having a higher surface tension compared to other liquids.

Water is an excellent solvent for polar molecules, including proteins, due to its ability to form hydrogen bonds. Proteins, which contain hydrogen bonding sites (such as O and N atoms), can readily form hydrogen bonds with water molecules. Compared to other polar solvents, water has a higher number of hydrogen bonds, allowing proteins to bind strongly to water molecules. As per the solubility data in the pharmacopeia, snakehead fish concentrate exhibits good solubility in water solvents (Soderberg and Johanna, 2013). It requires a ratio of 5 parts of distilled water (aquadest) to dissolve 1 part of snakehead fish concentrate.

Table I. Solubility of Snakehead Fish Concentrate in Aquadest

Replication	Weight of Soluble Concentrate
Replication 1	4,2 g
Replication 2	4 g
Replication 3	3,8 g
Average	4 g
SD	0,2

Ethanol Solvent

Ethanol is a commonly used semi-polar solvent that can form hydrogen bonds with other molecules. The solubility of substances in solvents is influenced by the presence of polar and nonpolar bonds. In the case of snakehead fish concentrate, it does not dissolve completely in ethanol solvent. Polar substances tend to dissolve in polar solvents, while nonpolar substances dissolve in nonpolar solvents. Proteins, being polar compounds, can exhibit some solubility in ethanol due to their ability to interact with polar solvents. However, the solubility of proteins in ethanol is generally not as good as in water solvents.

This is because ethanol is a semipolar solvent. Ethanol cannot dissolve proteins because it contains hydrocarbon bonds that cannot form hydrogen bonds. The low polarity of the hydrocarbon chain in ethanol prevents it from effectively binding to proteins (Soderberg and Johanna, 2013). The solubility percentage of snakehead fish concentrate in ethanol solvent is 0%. According to the pharmacopeia, snakehead fish concentrate has properties that make it difficult to dissolve in ethanol solvent, as it requires more than 100 parts of ethanol to dissolve 1 part of snakehead fish concentrate. Therefore, the solubility percentage of snakehead fish concentrate in ethanol solvent is 0%.

Table II. Solubility of Snakehead Fish Concentrate in Ethanol

Replication	Weight of Soluble Concentrate
Replication 1	0 g
Replication 2	0 g
Replication 3	0 g
Average	0 g
SD	0

Glycerin Solvent

The results indicated that snakehead fish concentrate was unable to dissolve in glycerin as a solvent. This could be attributed to the significant difference in the dielectric constants between glycerin and snakehead fish concentrate. Glycerin has a relatively lower dielectric constant compared to snakehead fish concentrate. Glycerin has a boiling point of 290°C, a melting point of 17.8°C, and a dielectric constant (KD) of 43.0 (Khusna, Irawan and Sari, 2015). The solubility percentage of snakehead fish concentrate in glycerin solvent is 0%.

Glycerin is indeed a polar solvent and is known to be hygroscopic, meaning it readily absorbs moisture from the air (Khusna, Irawan and Sari, 2015). Glycerin, being a hydrophilic molecule with a low molecular weight, can interact with proteins by forming hydrogen bonds with their reactive groups, especially when combined with water (Awwaly, Abdul and Esti, 2010). However, if glycerin is used as the sole solvent, it can potentially disrupt the protein structure due to its high boiling point. The solubility percentage of snakehead fish concentrate in glycerin solvent is 0%. According to the solubility standards in the pharmacopeia, snakehead fish concentrate is considered difficult to dissolve in glycerin solvent as it requires more than 100 parts of glycerin to dissolve 1 part of snakehead fish concentrate.

Table III. Solubility of Snakehead Fish Concentrate in Glycerin

Replication	Weight of Soluble Concentrate
Replication 1	0 g
Replication 2	0 g
Replication 3	0 g
Average	0 g
SD	0

Propylene Glycol Solvent

Snakehead fish concentrate shows good solubility in propylene glycol solvent, with 1.7 grams of concentrate dissolving in 20 ml of the solvent. The solubility of proteins is influenced by various factors such as molecular weight, amino acid composition, and protein conformation. Proteins contain both polar and nonpolar amino acids, resulting in hydrophilic and hydrophobic regions within the protein structure. The solubility of a protein is determined by the balance between its hydrophilic and hydrophobic characteristics. Propylene glycol, being a solvent and co-solvent, can enhance solubility and aid in dissolving proteins (Awwaly, Abdul and Esti, 2010).

Propylene glycol, classified as a third-class solvent with low toxicity, is a polar solvent that can effectively bind to polar protein compounds (Masyitoh, 2016; Dzakwan and Priyanto, 2019). The solubility of snakehead fish concentrate in propylene glycol solvent is 8.5%. Propylene glycol has a dielectric constant (KD) value of 33.0. Compared to glycerin, propylene glycol is a better solvent for proteins (Rowe, Sheskey, and Queen, 2009). This is because propylene glycol has a lower boiling point, allowing proteins to dissolve well without denaturation or coagulation that may occur at higher temperatures (>90°C). The average solubility percentage of snakehead fish concentrate in propylene glycol solvent is 8.5%. According to the pharmacopeia, snakehead fish concentrate can be dissolved in propylene glycol solvent, with a ratio of 11.76 parts of water to 1 part of snakehead fish concentrate. The resulting solution of snakehead fish concentrate in propylene glycol solvent appears clear.

Table IV. Solubility of Snakehead Fish Concentrate Propyleneglycol

Replication	Weight of Soluble Concentrate
Replication 1	1,72 g
Replication 2	1,66 g
Replication 3	1,73 g
Average	1,70 g
SD	0,03

Honey Solvent

Honey is composed of complex carbohydrates, water, and various minor components. Its main constituents are approximately 80-85% carbohydrates (glucose and fructose), 15% water, 0.1-4% protein, 0.2% ash content, and small quantities of amino acids, enzymes, vitamins, and other substances (Sjamsiah et al., 2018). Snakehead fish concentrate can dissolve up to 0.4 grams in 20 ml of honey solvent. This is likely due to the presence of water in honey, which aids in the solubility process of the concentrate. Snakehead fish concentrate is rich in protein. Protein solubility refers to the proportion of protein nitrogen (N) that can be dissolved under specific conditions. The interaction between water and protein takes place through peptide bonds in the polypeptide chain, as well as through dipole-dipole interactions and interactions involving polar, nonpolar, and ionized amino acids.

The physical properties of honey, such as its thick consistency and low water content, theoretically result in a low ability to dissolve concentrate or protein in honey. Protein is typically more soluble in water. Therefore, several experiments must be conducted to assess the solubility of the concentrate in honey before applying it to preparations (Bolontrade, Scilingo, and Añón, 2013). The average solubility percentage of snakehead fish concentrate in a honey solvent is 2%. According to pharmacopeial standards, snakehead fish concentrate is somewhat challenging to dissolve in honey solvent, as it requires 50 parts of honey to dissolve 1 part of snakehead fish concentrate.

Table V. Solubility of Snakehead Fish Concentrate in Honey

Replication	Weight of Soluble Concentrate
Replication 1	0,37 g
Replication 2	0,35 g
Replication 3	0,48 g
Average	0,4 g
SD	0,07

Manitol Solvent

Mannitol has a molecular weight of 182.17 grams/mol (Rowe, Sheskey, and Queen, 2009). It is a polar compound with high solubility in water (216 mg/ml at 25°C) and alkaline solutions, but it is difficult to dissolve in ethanol (Indonesia, 1979). Theoretically, mannitol has a low dielectric constant of 3.0, indicating nonpolar properties. Snakehead fish concentrate cannot be dissolved in a mannitol solvent because the low KD value of mannitol and its nonpolar nature are significantly different from the polar properties of snakehead fish protein. According to pharmacopeial standards, snakehead fish concentrate has low solubility in mannitol solvent, requiring more than 100 parts of mannitol to dissolve 1 part of snakehead fish concentrate.

Table VI. Solubility of Snakehead Fish Concentrate Manitol

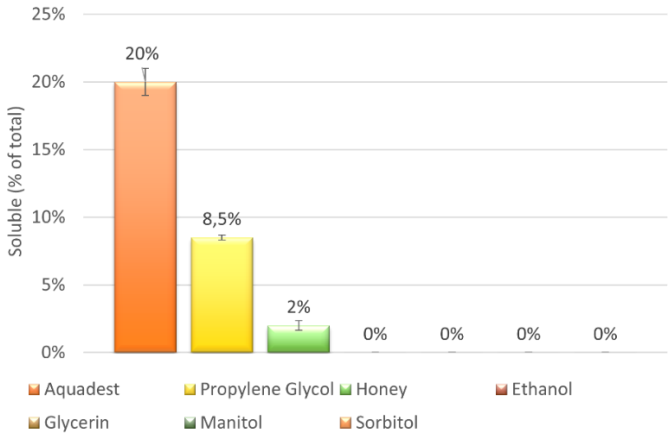
Replication	Weight of Soluble Concentrate
Replication 1	0 g
Replication 2	0 g
Replication 3	0 g
Average	0 g
SD	0

Sorbitol Solvent

Sorbitol is a powder with a higher relative humidity and a dielectric constant of 33.5. However, snakehead fish concentrate does not dissolve well in sorbitol solvent. While the KD value of sorbitol is similar to propylene glycol, the solubility results of sorbitol and propylene glycol in different concentrates may vary. This can be influenced by factors such as particle size, molecular size, temperature, pressure, solute and solvent properties, co-solvency, agitation, polarity, and polymorphs. The solubility percentage of snakehead fish concentrate in sorbitol solvent is 0%. According to pharmacopeial standards, snakehead fish concentrate has low solubility in sorbitol solvent, requiring more than 100 parts of sorbitol to dissolve 1 part of snakehead fish concentrate (Wahyuni, Erliza and Bonar, 2016).

Table VII. Solubility of Snakehead Fish Concentrate in Sorbitol

Replication	Weight of Soluble Concentrate
Replication 1	0 g
Replication 2	0 g
Replication 3	0 g
Average	0 g
SD	0



Picture 1. The Percentage Solubility of Snakehead Fish Concentrate

CONCLUSION

The solubility percentages of snakehead fish concentrate in various solvents are as follows: 20% in aquadest, 8.5% in propylene glycol, 2% in honey, 0% in ethanol, glycerin, mannitol, and sorbitol. According to pharmacopeial standards, snakehead fish concentrate is easily soluble in water, soluble in propylene glycol, slightly soluble in honey, and difficult to dissolve in ethanol, glycerin, mannitol, and sorbitol solvents. The addition of a cosolvent did not significantly increase the solubility of snakehead fish concentrate. Among the solvents tested, propylene glycol is considered the best solvent as it provides a clear appearance of solubility, followed by aquadest which gives cloudy and foamy results, and honey which gives concentrated results. Based on the solubility results, snakehead fish concentrate exhibits polar properties.

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