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OPTIMIZATION OF GOLDEN SNAIL (*POMACEA CANALICULATA*) MACERATION USING DIFFERENT SOLVENTS FOR ISOLATION OF TRITERPENOIDS

Optimasi Maserasi Keong Mas (*Pomacea canaliculata*) Menggunakan Pelarut Berbeda Untuk Isolasi Triterpenoid

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ABSTRACT

The golden snail is a type of mollusc pest that is very familiar to farmers in Indonesia. Utilizing the golden snail should be considered as an effort to reduce its impact as a plant pest and to increase its value in various uses. The golden snail is suspected to contain active triterpenoid compounds that have the potential to be used as a sex reversal agent in tilapia fish. These triterpenoid compounds can function as a synthetic hormone for 17α-methyl testosterone, which has been banned due to its environmental unfriendliness. The treatment with different solvents aims to determine the effect of solvent types on the characteristics of golden snail meat extract and to identify the best solvent between methanol (polar) and hexane (nonpolar). Maceration was carried out for 7 x 24 hours with a ratio of 1:3 (1 golden snail meat; 3 solvent). The filtered extract from the sample with methanol as a solvent was higher compared to N-Hexane sample. Conversely, the residue from the methanol extraction was relatively less than the residue from the N-Hexane extraction. The yield of the golden snail extract using methanol was 0.83 ± 0.36, while with N-Hexane it was 0.45 ± 0.35. Golden snail meat has a highwater content. Methanol is capable of dissolving polar compounds and can interact with water molecules through hydrogen bonding, which results in the formation of hydrogen bonds. During extraction, hydrogen bonds that bind active components in the snail meat, which also has a high moisture content, are extracted and dissolved in the methanol solvent. On the other hand, hexane is a relatively non-reactive solvent and mostly reacts with acids, bases, and reducing agents.

Keywords: Golden Snails; Maceration; Solvent; Triterpenoid; Yield

ABSTRAK

Keong mas adalah jenis hama moluska yang sangat dikenal oleh para petani di Indonesia. Pemanfaatan keong mas perlu dipertimbangkan sebagai upaya untuk mengurangi dampaknya

sebagai hama tanaman sekaligus meningkatkan nilai guna dalam berbagai pemanfaatan. Keong mas diduga mengandung senyawa aktif triterpenoid yang berpotensi digunakan sebagai agen pembalik kelamin (sex reversal) pada ikan nila. Senyawa triterpenoid ini dapat berfungsi sebagai hormon sintetis pengganti 17α-methyl testosterone, yang telah dilarang penggunaannya karena tidak ramah lingkungan. Perlakuan dengan berbagai jenis pelarut bertujuan untuk mengetahui pengaruh jenis pelarut terhadap karakteristik ekstrak daging keong mas dan untuk mengidentifikasi pelarut terbaik antara metanol (polar) dan heksana (nonpolar). Proses maserasi dilakukan selama 7 x 24 jam dengan perbandingan 1:3 (1 bagian daging keong mas; 3 bagian pelarut). Jumlah filtrat yang dihasilkan dari sampel dengan pelarut metanol lebih tinggi dibandingkan dengan sampel yang menggunakan pelarut N-heksana. Sebaliknya, residu dari ekstraksi dengan metanol relatif lebih sedikit dibandingkan residu dari ekstraksi menggunakan N-heksana. Rendemen ekstrak keong mas dengan menggunakan pelarut metanol adalah 0,83 ± 0,36, sedangkan dengan N-heksana adalah 0,45 ± 0,35. Daging keong mas memiliki kandungan air yang tinggi. Metanol mampu melarutkan senyawa polar dan dapat berinteraksi dengan molekul air melalui ikatan hidrogen, yang menghasilkan pembentukan ikatan hidrogen. Selama proses ekstraksi, ikatan hidrogen yang mengikat komponen aktif dalam daging keong yang juga memiliki kadar air tinggi akan terekstraksi dan larut dalam pelarut metanol. Sebaliknya, heksana adalah pelarut yang relatif tidak reaktif dan sebagian besar hanya bereaksi dengan asam, basa, dan agen pereduksi.

Kata Kunci: Keong Mas; Maserasi; Pelarut; Rendemen; Triterpenoid

INTRODUCTION

Golden snails (Pomacea canaliculata) are a type of mollusk that is very well known among farmers in Indonesia. This animal is often considered a pest for rice plants. The utilization of golden snails should be optimized as a step to reduce their impact as pests while increasing their economic value. Golden snails are thought to contain active compounds that have the potential to be used for the sex reversal process in tilapia (Noor *et al*., 2023). Sex reversal is a method that aims to direct the development of fish sex to the desired sex (Suseno *et al*., 2020).

This process is generally applied to female fish, which are considered less profitable for farmers. During the rearing stage, female tilapia often experience significant problems related to size differences, where their size is much smaller than male tilapia. This happens because of spontaneous spawning in heterosexual fish farming. The growth energy of female tilapia is used more for the spawning process, so that their body size becomes relatively small compared to males. This condition is clearly detrimental to farmers and requires action by directing the development of tilapia gonads to produce males. The masculinization process, often called maleization, is usually carried out using commercial products such as 17α-methyl testosterone. However, the use of this compound has been banned by the government because the compound is difficult to degrade in the environment.

Aromatase inhibitors work by inhibiting the formation of estrogen, thereby increasing testosterone hormone levels that encourage the development of male genitalia (Sarida *et al*., 2010). Golden apple snails are thought to contain active triterpenoid compounds that have the potential to function as aromatase inhibitors, which need further research. The active triterpenoid compound plays a role in activating macrophages, which then increases the process of phagocytosis and interleukin secretion. This interleukin secretion will stimulate cells to produce antibodies (Besung, 2009).

The process of isolating active compounds from snail meat is carried out through extraction stages, which include maceration, filtration, and evaporation. Separation of active compounds is an important step because it determines the amount or percentage of the resulting

yield. Extraction is a method of separating one or more components from solid or liquid material. This process begins by mixing the extraction material with the solvent, allowing contact between the materials at the interface, so that mass separation occurs through a diffusion mechanism (Ansel, 2008). According to Wijngaard *et al*., (2012), several factors that affect extraction using solvents include particle size, type of solvent, ratio of solvent to material, temperature, and duration of extraction.

The amount of yield produced determines the value of the extract (Armando, 2009). Therefore, testing various types of solvents is needed to obtain the best extract characteristics. This study aims to evaluate the effect of solvent types on the characteristics of golden snail meat extract and determine the most effective solvent to produce optimal extract. The study used methanol and n-hexane as solvents, each of which has a different level of polarity. Methanol is a polar solvent, while n-hexane is a nonpolar solvent.

RESEARCH METHODS

This study was conducted for eight weeks in September 2024 at the Fisheries Laboratory and Analysis Laboratory of the Lampung State Polytechnic. The extraction process begins by weighing the snails in shelled condition to record their initial weight. Next, the snail shells are broken, and the meat is separated and cleaned. The snail meat is then reweighed to obtain its net weight. Extraction of snail meat is carried out using the maceration method, which is soaking in different solvents. The snail meat is crushed with a blender until smooth, then soaked in methanol solvent for 7 x 24 hours with a ratio of 1:3 (1 part snail meat; 3 parts solvent) (Noor *et al*., 2023). Each treatment is carried out with three repetitions to ensure the accuracy of the results. After that, the soaking is filtered to separate the filtrate from the residue. The active compounds in the filtrate are then separated through the evaporation process using a rotary evaporator. Yield calculation:

The yield calculation is done by dividing the percentage of the extract weight and dry weight of the snails:

$$\text{Yield} = \frac{\text{extract weight}}{\text{Dry weight}} \text{ x } 100\%$$

Phytochemical testing to detect steroid and triterpenoid content is done by dissolving a sample weighing 0.1 grams into chloroform. After that, 0.5 ml of acetic anhydride and 2 ml of concentrated sulfuric acid are added. Indication of positive results can be seen from the change in color of the solution; red or purple at the border of the solution indicates the presence of triterpenoids, while blue or green indicates the presence of steroids (Sinulingga *et al.,* 2008).

RESULT

Pomacea canaliculata L. adalah hewan bertubuh lunak yang tergolong dalam kelas Gastropoda dan bergerak menggunakan bagian perutnya. Hewan ini memiliki ciri khas berupa cangkang berwarna kuning keemasan dengan bentuk bulat. Selain warna kuning, beberapa individu memiliki cangkang berwarna cokelat tua.

Figure 1. Golden snail research material

Maceration using different solvents produces dregs. The dregs produced by the sample with methanol solvent amounted to 93 grams, which is a value much lower than the weight of the snail meat before maceration of 166 grams, meaning that methanol is effective in absorbing chemicals and reducing the material to be drawn into the filtrate. Meanwhile, the dregs produced in the sample with N-Hexane solvent only experienced a slight reduction from the initial sample amount of 166 grams to 160.81 grams. This indicates that N-hexane does not have the ability to attract strong active ingredients, so the remaining dregs reduction value is still too high, even approaching the initial weight.

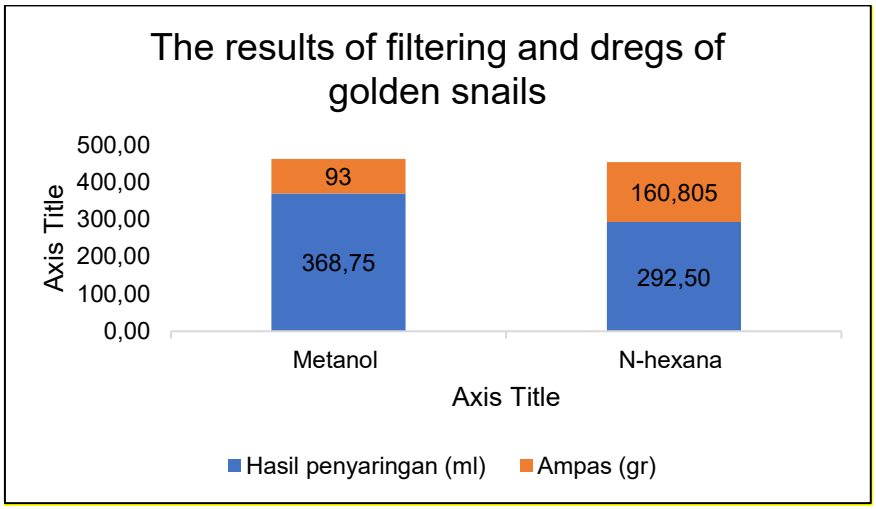

Figure 2. Graph of filtration results (filtrate) and dregs

The filtered filtrate after the maceration process was evaporated using a rotary evaporator to obtain a paste extract separated from the filtrate. The amount of extract produced by methanol solvent was 1.37gr while n-hexane was 0.75gr. The total extract results showed that the effectiveness of the solvent played a major role in attracting chemical compounds found in snail meat.

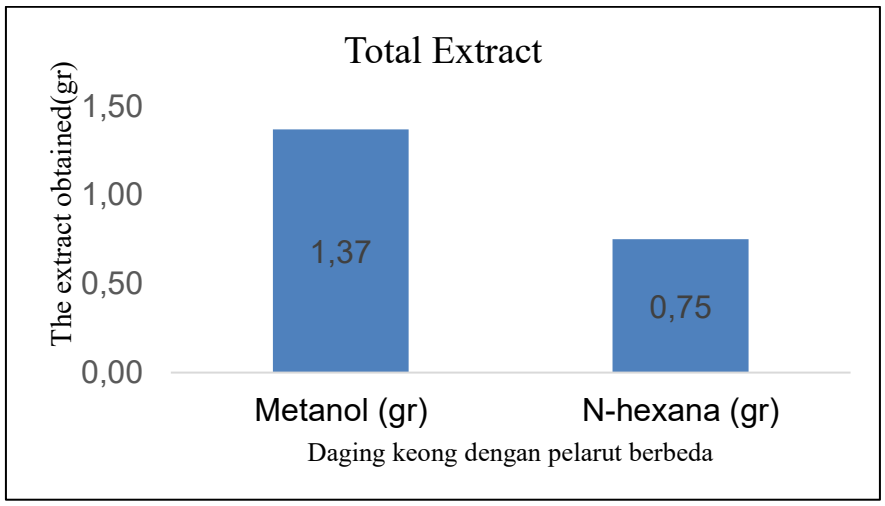

Figure 3. Total Extract

The yield results show that there is an influence of the type of solvent used on the amount of yield produced. Several factors that affect the yield include the size of the simplicia, the type of solvent, the polarity level of the solvent, and the duration of maceration (Hidayanti *et al*., 2017). The golden snail extract using n-hexane solvent produced a yield of 0.45%, while using methanol solvent produced a yield of 0.83%. The higher yield in methanol is thought to be due to its polarity, which is able to dissolve more bioactive components. Research by Houghton and Raman (1998) supports this finding, which states that polar solvents such as methanol can extract phenolic compounds, steroids, terpenoids, alkaloids, and glycosides. In contrast, n-hexane solvent, which is nonpolar, only dissolves a few bioactive components.

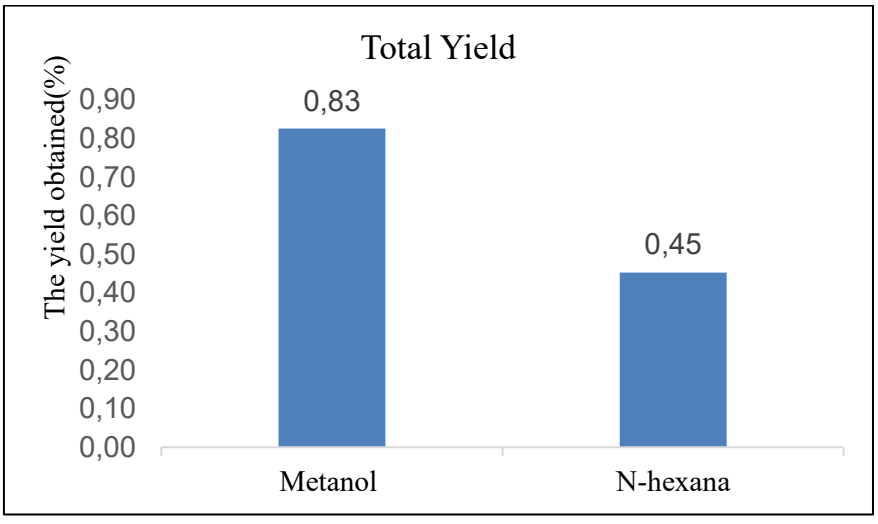

Figure 4. Total yield

DISCUSSION

Methanol has the ability to dissolve polar compounds and can attract water molecules bound through hydrogen bonds. During the extraction process, the hydrogen bonds that bind the active components in the snail meat tissue, which has a high water content, will break down and dissolve in methanol solvent (Meydia, 2016). In contrast, n-hexane is a relatively less reactive solvent and generally only reacts with acidic, basic, or reducing agent compounds.

Yield (different solvents)

Specific gravity is defined as the ratio between the density of a substance and the density of water, calculated based on the mass per unit volume. The measurement of specific gravity aims to determine the value of mass per unit volume as a specific parameter in liquid extracts to become thick extracts that can still be poured. In addition, specific gravity is also related to the level of purity of the extract from possible contamination (Ministry of Health of the Republic of Indonesia, 2000).

Yield is defined as the ratio between the weight of the material used, namely the weight of pure extract from golden snail meat, to the initial weight of the snail meat that was extracted. The results of the yield of golden snail extract based on the average value of three replicate samples in each solvent can be seen in Figure 4.

The duration of soaking the material in the solvent also affects the amount of extract produced, where longer maceration times tend to produce greater yields. This is in accordance with the research of Cikita et al., (2016), which concluded that the longer the extraction time, the higher the yield obtained. This condition occurs because the contact between the solvent and the raw material lasts longer, allowing the dissolution process to continue until the solvent reaches its saturation point with the raw material.

Utilization of Triterpenoids-Sex reversal

The active compound that is the main focus in this study is triterpenoid. Triterpenoid extracted from Pegagan leaves is known to have the potential to be used as a sex reversal ingredient in Guppy fish (Adibtyawan, 2016). The results of quantitative tests of golden snail extract showed that this extract contains triterpenoids, which is indicated by the appearance of a purplish red color indicating the presence of triterpenoids (Saha *et al*., 2011). Therefore, the triterpenoids detected in the golden snail extract in this study need to be tested further by applying to test animals to assess their effectiveness in the fish masculinization process.

According to research by Golan *et al*., (2008), triterpenoid saponins have the potential as aromatase inhibitors, which have been shown to provide significant results in the masculinization process. Priyono *et al*., (2013) explained that aromatase is an enzyme that catalyzes the conversion of testosterone (androgen) to estradiol (estrogen). Aromatase inhibitors function to inhibit the formation of the aromatase enzyme in the steroidogenesis process, which leads to the formation of testosterone and supports the development of male genitalia. Research by Sarida *et al*., (2010) also supports this, stating that the P-450 enzyme inhibits the formation of estrogen and increases testosterone production which supports male genital differentiation. Aromatase activity is localized in the brain, which affects behavioral control in the ovaries, affects follicle maturation and ovulation rates (Silverine *et al*., 2000).

Aromatase activity is related to gonadal structure, where low aromatase activity in larvae tends to result in testicular formation, while high aromatase activity leads to ovary formation (Scholz and Gutzeit, 2000). Aromatase inhibitors work by inhibiting aromatase activity in estrogen synthesis. This inhibition causes a decrease in estrogen concentration, which leads to inactivation of aromatase gene transcription as feedback (Balthazrtdan Ball, 1989 *in* Server *et al.,* 1999).

CONCLUSION

The extraction yield using methanol has a higher yield (0.83%) compared to n-hexane solvent (0.45%). While the total methanol extract is 1.37 grams and 0.75 grams for N-hexane solvent. Thus, methanol solvent is more effective in extracting triperpenoid active ingredients from snail meat.

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