PROCEEDINGS

16th ASEAN Regional Symposium on Chemical Engineering

"Chemical Engineering at the forefront of Global Challenges"

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FOREWORD

It is with great pleasure that the Chemical Engineering Department of the University of Santo Tomas (UST), on the occasion of its 75th Foundation Anniversary, hosts the 16th ASEAN Regional Symposium on Chemical Engineering (RSCE), in cooperation with the Philippine Institute of Chemical Engineers (PIChE) and the PIChE-Metro-Manila Academe Chapter. As UST prepares to celebrate its Quadricentennial in 2011, it opens its doors to the members of the Chemical Engineering Scientific and Professional Community in the ASEAN and Asia-Pacific regions in the spirit of international cooperation and collaboration in order to bring forth technological solutions to pressing global challenges. Thus, the theme of the 16th ASEAN RSCE is *"Chemical Engineering at the Forefront of Global Challenges"*.

As this is the first time that the Chemical Engineering Department is hosting an international event as prestigious as the 16th ASEAN RSCE, it is keen in keeping the Symposium as a venue for meaningful scientific and professional exchanges among its participants and ensuring that such exchanges become a catalyst of collaboration and innovation in the ASEAN Region. Therefore, in addition to the customary Technical and Poster Sessions, four fora were included in the Scientific Programme where topics that are deemed apt in addressing current regional and global challenges are tackled. It is hoped that this format will accommodate Academic, Industrial and Governmental viewpoints, both in highly scientific discussions and more informal exchanges of ideas, and more effectively synthesize sustainable solutions to pressing global challenges. This Proceeding is a testament of such exhilarating discussions and exchanges.

The Organizing Committee recognizes how the Chemical Engineering discipline is evolving into an almost basic science-like discipline on which allied fields anchor to create new branches of technological disciplines of highly specific applications. It is viewed that this evolution reiterates the fact that Chemical Engineering will continue to be a highly relevant discipline in responding to global challenges that involve science and technology. Therefore, this year, rather than dividing the Technical Sessions according to Chemical Engineering Principles, the Organizing Committee divided the Scope of the Symposium into five areas where different Chemical Engineering principles may be used to address specific issues that require technological intervention: (1) Emerging Technologies, (2) Materials Innovation, (3) Processing: State of the Art, (4) Product Innovation and Entrepreneurship, and (5) Advances in Chemical Engineering Education. More than 100 paper and poster presentations with authors from 15 different countries are showcased in the 16th ASEAN RSCE. We are grateful for their participation and the opportunity to host them. Likewise, the Organizing Committee thanks the invited speakers, Technical Session Chairs and all the attendees. The Organizing Committee also wishes to thank the 16th ASEAN RSCE sponsors, donors and exhibitors, without whose generosity, holding this event will not be possible.

The historical Manila Hotel was chosen as a venue because of its long history of catering to people and events that had significant impact to the Philippines. The 16th ASEAN RSCE, being a milestone for the UST Chemical Engineering Department, deserves to be held in a storied venue such as the Manila Hotel.

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UST History

The University of Santo Tomas (UST) is the oldest existing university in Asia and in terms of student population, the largest Catholic University in the world located in one campus. It was through the pioneering desire of Bishop Miguel de Benavides, O.P., (1550-1605), the third Archbishop of Manila, to establish an educational institution and the donation he bequeathed, that the University of Santo Tomas was founded on April 28, 1611. It was originally conceived as a school to prepare young men for the priesthood. Originally located within Intramuros, the Walled City, UST was first called Colegio de Nuestra Señora del Santisimo Rosario and was later renamed Colegio de Santo Tomas, in memory of the foremost Dominican theologian, St. Thomas Aquinas.

The University holds three distinct titles: 'Royal' granted by King Charles III of Spain in 1795, 'Pontifical' bestowed by Pope Leo XIII on September 17, 1902, and 'The Catholic University of the Philippines' conferred by Pope Pius XII in 1947.

It transferred to its present location in España, Sampaloc, Manila in 1927.

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Comparison of Different Extraction Techniques for Isolation of Testosterone from Sea Cucumber (*Holothuria scabra J*)

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ABSTRACT: Testosterone, the steroid hormone, is not only produced by sea cucumber but a lot of other organisms as well, which is used to sex reversal and aphrodisiac. This research aimed to comparing the conventional extraction methods for isolation of testosterone from sea cucumber. The solvent is selected from previous study on reflux extraction with solvent of acetone, methanol, methanol- chloroform mixture (1:2) and chloroform solvent. Ratio of material: solvent are 1:1, 1:2 and 1:3 respectively. The highest result is obtained by successively use methanol: chloroform, acetone, chloroform and methanol mean equal to 0.2728 mg / 100g dry weight (dw), 0.2623 mg/100 g (dw), 0.1606 mg/100g (dw) and 0.0920 mg/100 g (dw). Influence of ratio showed increasing usage of solvent increase the result of extraction. The highest materials: solvent ratio is obtained at ratio 1:3, 1:2 and 1:1. The highest extraction in maceration extraction is obtained by using acetone at ratio 1:3 equal to 0.07721 mg/100 g dry weights

Keywords: testosterone, sea cucumber, maceration, soxhlet, reflux

INTRODUCTION

One of marine resources that has high value and potential to become biopharmaceutics is sea cucumber (*Holothuria scabra J*) which is also known as teat fish, sandfish and sea ginseng. Currently, sea cucumber in Indonesia is exported in dried form (*beche-de-mer*), *konoko* (dried gonad), *konowata* (salted intestines) (Martoyo *et al.* 2004). This product is popular as food for health because it can increase men's vitality due to the testosterone content (Kustiariah. 2006). Utilization of extraction as aphrodisiac on human has been done and tested on mice, as an ingredient for sex reversal on commodities where male has more economical value than female such as on lobster and gapi fish (Nurjanah, 2008). Economically, sea cucumber has two important values, which are as marine biopharmaceutical source and as food for health. In addition, sea cucumber is also used as raw material in various industries in China (Kerr, 2000). Chemical contents of wet sea cucumber consist of 44-55% protein, 3-5% carbohydrate and 1.5% fat. According to Fredalina, important fatty acid contents in sea cucumber such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are involved in wound healing agent and anti-thrombotic (Fredalina *et al*. 1998). In addition, sea cucumber also contains antibacterial active ingredients (Haug *et al*. 2002; Villasin & Christopher, 2000; Ridzwan, 2005), antifungal (Muray, A. P. 2002), anticoagulant (Mulloy, 2000), as protease producer (Xue-Yan Fu *et al*. 2005a) and arginine kinase (Xue-Yan Fu *et al*. 2005b).

Problem in producing steroid from sea cucumber extraction is the low yield of extraction, thus sea cucumber has not yet been utilized in industrial scale biopharmaceutical source for fulfilling natural testosterone need. Correct extraction method will produce high steroid content (Goat & Toshihiro, 1997). This paper emphasize research on factors which affect conventional extraction, such as the conventional methods (maceration,soxhlet and reflux), solvent type, and material : solvent ratio.

MATERIALS AND METHODS

Characterization of Sea Cucumber

Early stage of the research was characterizing the sea cucumber to be extracted including type and age of sea cucumber based on characteristics and criteria (length and weight) which reflect the age of the sea cucumber. Raw material used was matured sea cucumber (*Holothuturia scabra J*) caught by the fishermen in Bengkulu province (Indonesia) weighing 200-500 grams each. The characteristics were the same as a sea cucumber (Wibowo *et al*. 1997). The characteristics of sea cucumbers used in the research were the same as the characteristics of the steroid producing sea cucumbers identified by Riani *et al* (2007) and also the same as sea cucumbers used by Kustiariah (2005) and Nurjanah (2008).

Chemical analysis of Sea Cucumber

Suitable sea cucumber fulfilling the criteria was cleaned and the flesh was separated from the entrails, washed and milled. Then proximate analysis was carried out by using ether, NaOH, H_2SO_4 and alcohol.

Conventional Extraction

***Maceration extraction*.** Fresh sea cucumber that has been milled was weighed 100 gr, put into erlemeyer, soaked in several type of solvents and several ratio (material:solvent) and then put into refrigerator for 24 hours.

***Soxhlet extraction*.** Fresh sea cucumber that has been milled was weighed 100 gr, wrapped by using filtering paper and then put in sampling container on soxhlet equipment. Heating device of the equipment was set at 50^{o}C for 4 hours.

Reflux extraction. Reflux extraction is an extraction by soaking and heating the material and solvent simultaneously. Fresh sea cucumber that has been milled was weighed 100 gr, put into Erlenmeyer and soaked in solvent with several ratios. Chemicals used in conventional steroid extraction are methanol, acetone, methanol: chloroform and chloroform.

RESULTS AND DISCUSSION

Characterization of Sea Cucumber

The sea cucumbers were round in shape, long like a cucumber with the back were dark grey with white or yellow spots and whole body surface were covered by lime. The sea cucumbers bodies were rough, muscular with spots on the skin. An illustration of a sea cucumber used in the research is shown in **Figure 1**.

Figure 1. Steroid raw material of a sea cucumber (*Holuthuria scabra J*).

Conventional Extraction

Maceration extraction of sea cucumber

In sequence, the most polarized solvent used in the research was methanol, acetone, solvent mixture of methanol:chloroform and chloroform with dielectric constant of 32.6, 20.7, ±14.07 and 4.8 deybe respectively. Solvent's ability in extraction is very much determined by the suitability of polarization level of the material with the solvent. Polarized solvent will dissolve polarized components and non-polarized solvent can extract non-polarized components (Ashton, N.F and McDermott, C. 2004). Maceration extraction results can be seen in **Figure 2** in details.

According to Riddick and Burger (1970), lower dielectric constant of a solvent means the solvent is more polarized (Riddick J. A. and William B.B. 1970). Lowering dielectric constant from 32.6 (methanol) to 20.7 (acetone) shows improved result. However, lowering dielectric constant will decrease the amount of obtained result.

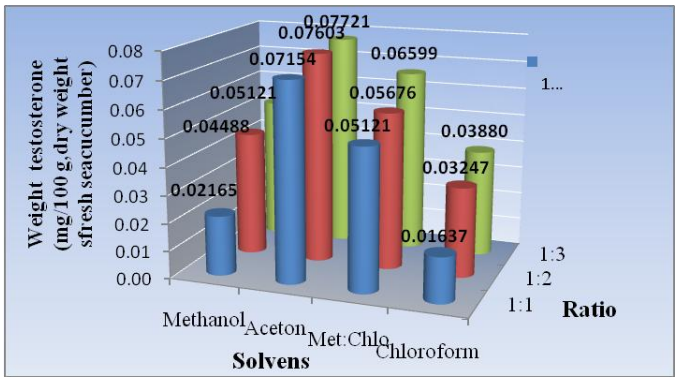

Figure 2. Sea cucumber's testosterone weight by maceration extraction.

Differentiation test showed that extraction result by using methanol, acetone, methanol: chloroform and chloroform solvent noticeably different at 5% level. This is due to differences in polarization level of the solvents affect the solvents ability to extract the material. Polarized solvent will dissolve polarized components and non-polarized solvent can extract non-polarized components (Agilera, 2003). Thus, it can be seen that sea cucumber's steroid differs from the common fats which are easily dissolved in non-polarized solvents. High solubility of sea cucumber's steroid in acetone (semi-polarized solvent) shows that sea cucumber is categorized as semi-polarized fat with –OH group at C-17.

Ratios effect of material:solvent in maceration extraction show the similarity of extraction characteristics for all kind of solvents, i.e. more solvent used or lower ratio will give more results obtained. Ratio of material:solvent which yields highest extraction is 1:3. In the case, more solvent caused higher contact between material and solvent, thus solubility was increased. At ratios of 1:2 and 1:1, solvent used was decreased; hence lowering the contact between them and the solubility was decreased.

Further testing showed that ratio 1:1 noticeably different from 1:3. This is because in maceration extraction which is grouped in batch system using single stage without mixing, contact between the material and solvent is only affected by the amount of solvent used (Tzia & Goerge, 2003). This result clarifies the contact between material and solvent in maceration extraction depends on the amount of solvent used. Nevertheless, solvent usage is limited by the contents of material compound and the cost of solvent with its effects on the environment. Maceration extraction produces highest extraction result by using acetone as solvent with material: solvent ratio at 1:3.

Soxhlet extraction of Sea Cucumber

Soxhlet extraction of sea cucumber by using soxhlet equipment showed fluctuating results as seen in **Figure 3**. This is due to the high water content (88.99%) of the fresh raw material, so that the water covered the solvent's surface in extraction result. The water layer blocked reflux process. At solvent ratio 1:1 and 1:2, water and solvent ratio was almost reaching ± 89 ml: 100 mL and ±89 mL:200 mL, circulation was very slow therefore the contact of material and solvent was very low. At solvent ratio 1:3, water and solvent ratio was ±89 ml:300 mL, therefore reflux process could occur. This shows that more solvent used will reduce water on the surface and hence lowering the blockage to solvent circulation, thus reflux cycle can occur normally.

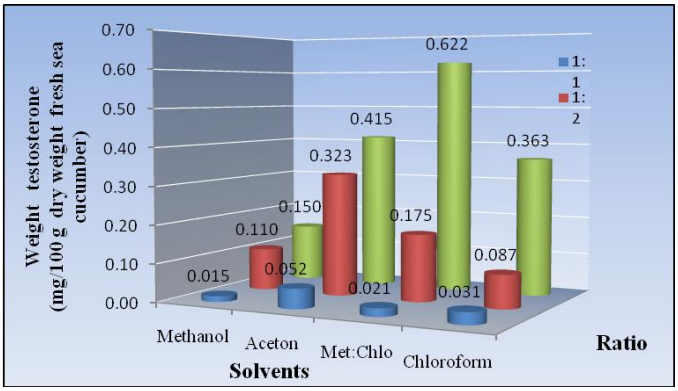

Figure 3. Sea cucumber's testosterone weight by soxhlet extraction.

In soxhlet extraction method, solvent's ability in extracting is not only determined by the suitability of polarization level of the solvent and material, but is also determined by solvent's boiling point. Methanol chloroform solvent is able to extract steroid more than acetone due to the boiling points of methanol chloroform is higher than acetone. The evaporation of methanol chloroform is not as fast as acetone with the solvent's circulation is slower and contact time is longer, therefore extraction is higher.Highest extraction was obtained by methanol chloroform compound solvent at material: solvent ratio of 1:3.

Reflux extraction of Sea Cucumber

Sea cucumber's steroid extraction by reflux is shown in **Figure 4**. Solvent's influence in reflux extraction showed highest result by using chloroform; solvent's mixture of methanol chloroform, acetone, and the lowest is methanol. Tests showed that by using chloroform with the result of 7.9547 mg/100 gr (dw) did not show significant difference from the result by using mixture of methanol chloroform which was 7.6142 mg/100 gr (dw).

Effects of material: solvent ratio showed that more solvent used produced higher extraction. However, further testing showed that ratio of 1:2 did not cause significant difference from 1:3. Therefore, the best solvent used in the next scale is the mixture of methanol chloroform with ratio of 1:2. The testing also showed that chloroform usage could be reduced, thus lowering the cost (chloroform is more expensive than methanol) and the environmental effects of chloroform could be reduced as well.

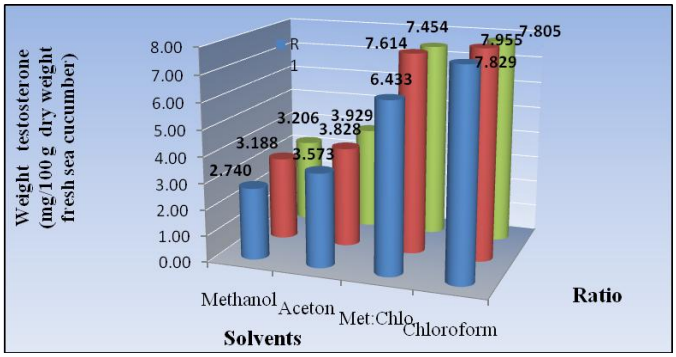

Figure 4. Sea cucumber's testosterone weight by reflux extraction.

Comparison

Comparison of extraction methods, solvents' type and material: solvent ratio can be seen in **Figure 5**. Highest extraction by maceration method was obtained by using acetone as the solvent with the material: solvent ratio of 1:3 and the result was 0.0772 mg/100 gr (dw). In soxhlet extraction, solvent mixture of methanol chloroform with ratio of 1:3 produced highest extraction of 0.6220 mg/100 gr (dw). While in reflux extraction, solvent mixture of methanol chloroform with the ratio of 1:2 produced highest extraction of 13.9547 mg/100 gr (dw)

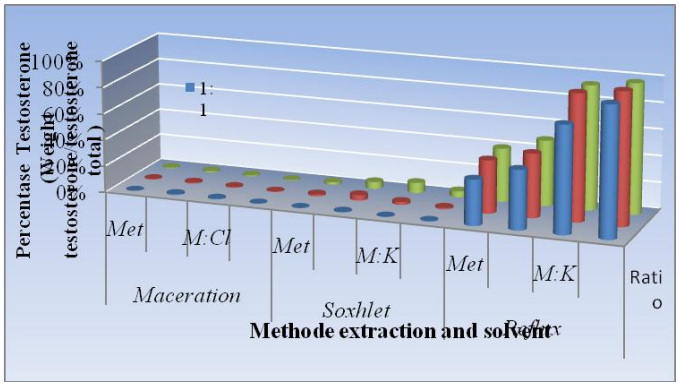

Figure 5. Testosterone weight comparisons

Further testing of the three extraction methods (maceration, soxhlet and reflux) showed significant differences, where extraction by reflux produced extraction containing highest testosterone, followed by soxhlet extraction and the lowest was maceration extraction. Differences in methods, solvents and ratio could improve results obtained from 0.97% (maceration) to 7.82% by soxhlet and even to 95.72% by reflux.

CONCLUSION

Effects of the solvent's type and material: solvent ratios were not the same in the extraction methods. However, the effects of solvent's type on every ratio showed the same trend. Lower dielectric constant of the solvent (more non-polarized) caused more testosterone extraction. This was due to the testosterone steroid extracted was in the group of semi-polarized fat. The effect of material: solvent ratio in maceration extraction showed that when more solvent was used, testosterone extraction was higher.

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