

EXTRACTION OPTION ETHANOL-WATER FOR EURYCOMANON CONCENTRATION ON PASAK BUMI ROOT WITH SIMPLEX LATTICE DESIGN METHOD

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ABSTRACT

Background: Eurycomanon an active substance from the roots of the pasak bumi used to treat cancer. The compounds present in very small amounts that need to be optimized solvent.

Objective: The purpose of this study is to optimize the extraction method of eurycoma or *pasak bumi* roots using simplex lattice design.

Methods: One gram of root powder macerated pasak bumi each in 5 ml of solvent, with 3 different solvents, namely: water, ethanol 50 %, 70 % and 96 % ethanol. Maceration levels determined by HPLC with fased silica gel 60 F₂₅₄ and phase motion methanol: chloroform (8: 2), with a maximum length of 301 nm.

Outcome Measured: Levels obtained from the laboratory scale and then put into the equation Simplex Lattice Design (SLD) by incorporating response levels obtained, it can be calculated price coefsien ab and ab. Value of Y (response) for each variation mixtures A and B is calculated, so that the profile can be described.

Results: From the results obtained, the optimum composition of the fluid eurycomanom quote from the roots of the earth peg in a semi industrial scale is 100 % water conformed to the equation, simplex lattice design with the equation $Y = 71693.153 [A] + 7766.246 [B] + 7721.57 [A] [B]$.

Conclusion: Prediction results obtained between response levels on eurycomanon laboratory scale with statistical analysis by t test showed differences are not significant, so the simplex lattice design equations that can be obtained be used to predict the optimum ratio of ethanol-water.

Keywords: Root Pasak Bumi (*Eurycoma longifolia*, Jack), Eurycomanon, Simplex Lattice Design

INTRODUCTION

Cancer is one of the major diseases causing death. In 2010, deaths from cancer in the United States reached 25 % increased 7 % over the last 10 tahn (Brooks, 2011). In Indonesia, ranked sixth cause of cancer death (Tjindarbumi and Mangunkusumo, 2002).one plant efficacious as anticancer there was the root of the *pasak bumi*. However, if the plant is consumed in excess can be nefrososik (Chan and Choo, 2002). This indicates the nature of the active compounds in the plant roots *pasak bumi* not only selectively against cancer cells (Bhat and Karim). Extract active compounds need to be very high because of its potential as an anticancer.

Plant roots contain several *pasak bumi* efficacious anticancer compounds. Compounds contained in the roots of the 9 - methoxycanthin dominant - gone (Rosli et al., 2009), eurycomalacton (Chan et al., 1992), squalene (Morita et al., 1993), as well as eurycomanon (Ang et al., 2002). These compounds have antimalarial effects (Kuo et al., 2004), antitumor (Nurhanan et al., 2005), and chemopreventive (Conscience, et al., 2009). This compound is acidic so it can interact the premises chitosan (Kumar et al., 2005) so that the formula will be obtained chitosan nanoparticle - targeting compounds of the roots of the *pasak bumi* anticancer anticancer properties that have a high selectivity. Eurycomanon levels in plants by only 0.5%. Based on these two needs to optimize extraction by solvent that produces most of the levels, Ethanol and Water is commonly used as solvent extract active compounds in the extracts of natural products industry. Comparison of ethanol and water need to be sought to determine the extract eurycomanon maximum yield.

METHODS

Making a series of standar dsolution

Standard eurycomanon much as 125 mg, dissolved in distilled water : methanol (1:1) to 25 ml (mother liquor = 5 mg / ml) made a series of standard solution of 5mg/ml; 4mg/ml; 3mg/ml; 2mg/ml; 1mg/ml. Standard solution is then injected into the HPLC

column, silica gel 60 F₂₅₄ with a mobile phase of methanol : chloroform (8:2). The next area is calculated and linear regression equation to be used as a standard curve.

Optimization of fluids with the method simplex lattice design

Weighed 1.0 grams of powdered root *pasak bumi*, as much as 3 times and enter separately into flacon. Powder used in this study advance lipid extract with petroleum ether. Furthermore, each flacon plus different solvents are: water, 50 % ethanol and 96 % ethanol as much as 5 ml and macerated for 20 hours while often shaken, filtered, then each matched to the volume of 5 ml in a pint flask. The drawback with the solvent flowed through the dregs to 5 ml. Parameter optimization results is the level of the active substance is eurycomanon. Determination of the active compounds was done by HPLC with a C - 18 stationary phase and mobile phase pH buffer - acetonitrile and comparison optimized first (Choo and Chan, 2002).

Eurycomanon levels obtained from each solvent and then analyzed using simplex lattice design method to determine the composition of the ethanol - water solvent optimum eurycomanon to sum up the *pasak bumi* roots. Parameter optimization results is the level of the active substance is eurycomanon. Determination of the active compounds was done by HPLC with a C - 18 stationary phase and mobile phase pH buffer - acetonitrile and comparison optimized first (Choo and Chan, 2002).

RESULTS AND DISCUSSION

Preparation of the main ingredients

The main materials used in this study are the root of the *pasak bumi* Kalimantan. In a subsequent sample processing used three types of solvents or liquids that extract for laboratory scale is water, 50% ethanol and 96% ethanol, while for semi industrial scale using 50% ethanol, 70% ethanol and 96% ethanol by maceration method. Prior to extract eurycomanon in the sample, first performed lipid extraction samples with petroleum ether. This is to sum up the fat and

other non-polar compounds so it will not interfere with subsequent assays eurycomanon. Once the samples are free of fat and completely dry and free of residual petroleum ether, then made extract eurycomanon with different solvents using the maceration method. Eurycomanon defined in this study is hydrolyzed eurycomanon counts as eurycomanon.

Identification Eurycomanon

Identification eurycomanon qualitatively performed to determine the presence of eurycomanon in samples tested. If proven the existence eurycomanon using qualitative identification can then proceed to perform the assay eurycomanon in samples with three kinds of solvents or liquids extraction are water, ethanol 50% and 96% ethanol by maceration using HPLC. Identification eurycomanon scanning the HPLC used as a comparison compound eurycomanon and maximum wavelength measurements on samples of the *pasak bumi* roots, so that has been known to be able to identify whether the same sample obtained with reference compounds. Provided that eurycomanon more extract in 100% water. Calculation of each solvent eurycomanon levels using standard curves that exist on the same plate. Extraction eurycomanon levels that using ethanol 96%, is 1.45%, levels that extraction eurycomanon with ethanol 50%, which is 1.62% and the extraction eurycomanon levels with water, which is 1.39%. When the calculation is based on simplex lattice design formulated with:

$$Y = a [A] + b [B] + ab [A] [B]$$

With A = water and B = ethanol

For A = water 100%, B = ethanol 0;

$$Y = 71693.153;$$

$$Y = a[A] + b[B] + ab[A][B]$$

$$71693,153 = a[1] + b[0] + ab[1][0]$$

$$a = 71693,153$$

For A=water 0; B=ethanol 100%;

$$Y = 7766,246$$

$$Y = a [A] + b [B] + ab [A] [B]$$

$$7766,246 = a[0] + b[1] + ab[0][1]$$

$$b = 7766,246$$

For A=water 50%; B=ethanol 50%;
 $Y = 37799,307$

$$Y = a[A] + b[B] + ab[A][B]$$

$$37799,307 = a[0,5] + b[0,5] + ab[0,5][0,5]$$

$$37799,307 = \{71693,153[0,5]\} +$$

$$\{7766,246[0,5]\} + 0,25ab$$

$$ab = 41660,092$$

SLD equation obtained is:

$$Y = 71693,153 [A] + 7766,246 [B] +$$

$$7721,57[A][B]$$

The results of calculations by the content acquisition solution of water and ethanol are shown in Table. I. From Table I seen that the highest levels of eurycomanon obtained on 100 % water. Prediction Results Calculation and Experimental Results Simplex Lattice Design to determine the validity of the results of equations simplex lattice design on a laboratory scale so that the equation can be used on a different formula, then analyzed on a semi- industrial scale to 50% ethanol, 70% ethanol and 96% ethanol with the response simplex lattice design results obtained on a scale laboratory. The analysis is using SPSS by t test at 95% of confidence level. From the t-test results are obtained, the response levels eurycomanon extracted with 50% ethanol, 70% ethanol and 96% ethanol in equation simplex lattice design gained significance on 0.16. This shows that equation simplex lattice design on a laboratory scale calculations can be used to predict the ratio of ethanol - water.

Table I. Eurycomanon levels results maceration with water and ethanol solvent

A (Water)	B (Ethanol)	Average Level (µg/ml)
0	1	7.766,246
0,1	0,9	14.853,878
0,2	0,8	21.786,987
0,3	0,7	28.565,802
0,4	0,6	35.190,186
0,5	0,5	41.660,092
0,6	0,4	47.975,567
0,7	0,3	54.136,601
0,8	0,2	60.143,273
0,9	0,1	65.995,266
1	0	71.693,153

CONCLUSION

Based on the calculation method of simplex lattice design the most optimal solvent to sum up the roots of the *pasak bumi* eurycomanon is solvent water 100 % .

ACKNOWLEDGEMENT

Thank you submitted to Higher Education for funding this research through funding COMPETITIVE GRANTS fiscal year 2013/2011.

REFERENCES

- Ang, H. H., Hitotsuyanagi, Y., Fukaya, H., and Takeya, K., 2002, Quassinoids from *Eurycoma longifolia*, *Phytochemistry*, 59, (8), 833-837.
- Amri Q, 2012, Produksi Udang Cukup untuk Bahan Baku Industri, Indonesia finance Today, 12 Januari 2012.
- Bhat, R., and Karim, A. A., Tongkat Ali (*Eurycoma longifolia* Jack): A review on its ethnobotany and pharmacological importance, *Fitoterapia*, 81, (7), 669-679.
- Bourtoom, T., and Chinnan, M. S., 2008, Preparation and properties of rice starch-chitosan blend biodegradable film, *LWT-Food science and Technology*, 41, (9), 1633
- Brooks, M., 2011, 2010 Cancer Incidence In The Usa, <http://www.articlesnatch.com/Article/2010,Cancer-Incidence-In-The-Usa/2170908>, accessed in March 2011
- Calvo, P., Remunan-Lopez, C., and Vila-Jato, JL, 1997a, Chitosan and Chitosan / Ethylene Oxide Propylene Oxide Block Copolymer Nanoparticles as Novel Carriers for Proteins and Vaccines, *Pharm. Res.*, 14:1431-1436.
- Calvo, P., Remunan-Lopez, C. and Vila-Jato, JL, 1997b, Novel Hydrophilic Chitosan-Polyethylene Oxide Nanoparticles as Protein Carriers, *J. Appl. Polymer Sci.*, 63: 125-132.
- Chan, K. L., and Choo, C. Y., 2002, The toxicity of some quassinoids from *Eurycoma longifolia*, *Planta Medica-Natural Products and Medicinal Plant Research*, 68, (7), 662-663
- Chan, K. L., Iitaka, Y., Noguchi, H., Sugiyama, H., Saito, I., and Sankawa, U., 1992, 6i ± Hydroxyeurycomalactone, a quassinoid from *Eurycoma longifolia*, *Phytochemistry*, 31, (12), 4295-4298
- Choo, C. Y., and Chan, K. L., 2002, High performance liquid chromatography analysis of canthinone alkaloids from *Eurycoma longifolia*, *Planta medica*, 68, (4), 382-384
- Cho K, Wang X, Nie S, 2008, Therapeutic Nanoparticles for Drug Delivery in Cancer, *Clin Cancer Res* 1316,14:1310.
- Danhier, F, Feron, O, Preata, V, 2010, To Exploit the Tumor Microenvironment: Active and Passive Tumor Targeting Ofnanocarriers for Anti-Cancer Drug Delivery, *Journal of Controlled Release*, 148:135, 146
- Darusman, LK, 2005, Review: Roots Pasak Bumi (*Phaleria macrocarpa*) As Anti-Cancer, Patents February 14, 2005 Registration Number P00200500077
- De Padua, L.S., Bunyapraphatsara, N. and Lemmens, RHMS, 1999, Plant Resources of South East Asia. Medical and Poisonous Plants. Printed in Bogor. Indonesia (PROSEA), pp. 36, Backhuys Publishers, Leiden
- Fisher, DE, 1994, Apoptosis in Cancer Therapy: Crossing the Threshold, *Cell*, 78: 539-542
- Gibbs, JB, 2000, Anticancer Drug Targets: Growth Factors and Growth Factor Signaling, *J.Clin Invest*, 105, 109-111
- Hyo-Kyung, H. and Amidon, GL, 2000, Targeting Prodrug Design to Optimized Drug Delivery, *AAPSPharmScitech*, 2 (1) article 6.
- Johnson, JD, Ryan, MJ, Toft, JDII, Graves, SW, Hejtmancik, MR, Cunningham, M. L., Herbert, R.A., and
- Kamal, M., 2000. Two-year toxicity and carcinogenicity study of methyleugenol in F344 / N rats and B6C3F1mice, *Journal of Agricultural and Food Chemistry* 48 (8): 3620-3632
- King, RJB, 2000, *Cancer Biology*, 2nd ed., Pearson Education Limited, London
- Knapczyk, J., Krowczynski, L., Krzck, J., Brzeski, M., Nimberg, E., Schenk, D.,

- and Struszyk, H., 1989, Requirements of chitosan for Pharmaceutical and Biomedical Applications, in Skjak-Braek, G., Anthonsen, T., and Sandford, P. A., Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications; case 657-663, Elsevier: London
- Krauland, A.H. and Alonso, MJ, 2007, Chitosan Cyclodextrin Nanoparticles As Macromolecular Drug Delivery System, *Int. J. Pharm.*, 340: 134-142
- Kumar V and Banker G, 1995, Targeted Drug Delivery System, in Rhode Banker G and T, *Modern Pharmaceutics*, Marcel Dekker, New York.
- Kumar, M. N. V., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., and Domb, A. J., 2005, Chitosan chemistry and pharmaceutical perspectives, *ChemInform*, 36, (11), no-no
- Kuo, P.-C., Damu, A. G., Lee, K.-H., and Wu, T.-S., 2004, Cytotoxic and antimalarial Constituents from the roots of *Eurycoma longifolia*, *Bioorganic & Medicinal Chemistry*, 12, (3), 537-544
- Lin YS, Tungpradit R, Sinchaikul S, An FM, Liu DZ, Phutrakul S, Chen ST, 2008, The Delivery Of Targeting glycan-Based Paclitaxel prodrugs To Cancer Cells Via Glucose Transporters, *J Med Chem*. 51 (23) :7428, 7441.
- Morita, H., Kishi, E., Takeya, K., Itokawa, H., and Iitaka, Y., 1993, Squalene derivatives from *Eurycoma longifolia*, *Phytochemistry*, 34, (3), 765-771
- Mourya, VK, Nazma, E., and Inamdar, N., 2009, Trimethyl Chitosan and its Applications in Drug Delivery, *J Mater Sci: Mater Med*, 20: 1057-1079, Springer Science + Business Media, LLC.
- Minko T, Dharap SS, Pakunlu RI, Wang Y, 2004, Molecular Targeting Of Drug Delivery Systems To Cancer, *Curr Drug Targets*, 5 (4) :389-406.
- Neto, C. G. T., Dantas, T. N. C., Fonseca, J. L. C., and Pereira, M. R., 2005, permeability studies in chitosan membranes. Effects of crosslinking and poly (ethylene oxide) addition, *Carbohydrate research*, 340, (17), 2630-2636
- Nurhanan, M. Y., Hawariah, L. P., Ilham, A. M., and Shukri, M. A., 2005, Cytotoxic effects of the root extracts of *Eurycoma longifolia* Jack, *Phytotherapy Research*, 19, (11), 994-996
- Onishi, H. and Machida, Y., 1999, Biodegradation and Distribution of Water-Soluble Chitosan in Mice, *Biomaterials*, 20: 175-182.
- Rosli, N., Maziah, M., Chan, K., and Sreeramanan, S., 2009, Factors affecting the accumulation of 9-methoxycanthin-6-one in callus cultures of *Eurycoma longifolia*, *Journal of Forestry Research*, 20, (1), 54-58
- Shapiro, G.I., and J. Wade Harper, W., J., 1999, Anticancer drug targets: cell cycle and checkpoint control, *The Journal of Clinical Investigation*, 104 | (12), 1645-53
- Singla, A.K. and Chawla, M., 2001, Chitosan: Some Pharmaceutical and Biological Aspects-an Update. *J. Pharm. Pharmacol*, 53: 1047-1067.
- Shu, X. Z., and Zhu, K. J., 2000, A novel approach to prepare tripolyphosphate / chitosan complex beads for controlled release drug delivery, *International Journal of Pharmaceutics*, 201, (1), 51-58
- Shu, X. Z., and Zhu, K. J., 2001, Chitosan / gelatin microspheres prepared by modified emulsification and ionotropic gelation, *Journal of Microencapsulation*, 18, (2), 237-245
- Suh, J.K.F. and Matthew, HWT, 2000, Application of Polysaccharide Chitosan-Based Biomaterials in Cartilage Tissue Engineering: A Review, *Biomaterials*, 21: 2589-2598.
- Tiyaboonchai, W. 2003 Chitosan Nanoparticles: A Promising System for Drug Delivery, Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand, *Naresuan Univ. J.*, 11 (3) 51-66.
- Tjindarbumi, D. and Mangunkusumo, R., 2002, Cancer in Indonesia, Present and

- Future, Jpn. J. Clin. Oncol., 32 (suppl 1): S17-S21.
- Torchilin VP. 2007, Targeted Pharmaceutical Nanocarriers for Cancer Therapy and Imaging. AAPS Journal.; 9 (2) Article 15.
- Van der Merwe, S. M., Verhoef, J. C., Verheijden, J. H. M., Kotze, A. F., and Junginger, H. E., 2004, Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs, European journal of pharmaceutics and Biopharmaceutics, 58, (2), 225-235