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APPENDIX

APPENDIX A PERCENTAGE OF YIELD

The percent yield of *A. racemosus roots* extract is calculated by using this equation:

% yield = [dried extract weight (g) / plant material sample weight (g)] x 100

1. **% yield of AR 1-H extract**

   % yield = [1.03 (g) / 1.8 (Kg)] x 100

   = 0.06

2. **% yield of AR 1-E extract**

   % yield = [115.35 (g) / 1.8 (Kg)] x 100

   = 6.41

3. **% yield of AR 1-A extract**

   % yield = [87.65 (g) / 1.8 (Kg)] x 100

   = 4.87

4. **% yield of AR 1-B extract**

   % yield = [34.72 (g) / 1.8 (Kg)] x 100

   = 1.93

5. **% yield of AR 1-S extract**

   % yield = [87.45 (g) / 1.8 (Kg)] x 100

   = 4.86

6. **% yield of AR 2-H extract**

   % yield = [0.46 (g) / 1.8 (Kg)] x 100

   = 0.05

7. **% yield of AR 2-E extract**

   % yield = [57.29 (g) / 1 (Kg)] x 100

   = 5.73

8. **% yield of AR 2-A extract**

   % yield = [55.12 (g) / 1 (Kg)] x 100

   = 5.51

9. % yield of AR 2-B extract

% yield = [23.37 (g) / 1 (Kg)] x 100

= 2.34

10. % yield of AR 2-S extract

% yield = [72.85 (g) / 1 (Kg)] x 100

= 7.29

11. % yield of AR 3-H extract

% yield = [7.43 (g) / 4.8 (Kg)] x 100

= 0.15

12. % yield of AR 3-E extract

% yield = [525.07 (g) / 4.8 (Kg)] x 100

= 10.94

13. % yield of AR 3-A extract

% yield = [247.16 (g) / 4.8 (Kg)] x 100

= 5.15

14. % yield of AR 3-B extract

% yield = [115.54 (g) / 4.8 (Kg)] x 100

= 2.41

15. % yield of AR 3-S extract

% yield = [215.56 (g) / 4.8 (Kg)] x 100

= 4.49

16. % yield of AR 3-En extract

% yield = [32.4 (g) / 150 (g)] x 100

= 1.22

APPENDIX B PREPARATION OF STERILE MEDIA FOR YEAST

1. Sabouraud dextrose agar (Becton dickinson)

Pancreatic digest of casein	5.0	g
Peptic digest of animal tissue	5.0	g
Dextrose	40	g
Agar	15	g

Suspend the mixture 65 g in 1 lit of water, and autoclave at 121 ºC 15 minute. Cool the sterile media to <50°C before pipette media 20 ml in to sterile plastic disc (10 cm). Dry plates 1 days at room temperature, or 30 min with the lids slightly off at 37°C or in a laminar flow hood. Store dried plates wrapped at 4°C.

2. Sabouraud dextrose agar (Becton dickinson) + 1% olive oil

Pancreatic digest of casein	5.0	g
Peptic digest of animal tissue	5.0	g
Dextrose	40	g
Agar	15	g
Olive oil	1%	v/v

Suspend the mixture 65 g in 1 lit of water add 1% v/v of olive oil, and autoclave at 121 ºC 15 minute. Cool the sterile media to <50°C before pipette media 20 ml in to sterile plastic disc (10 cm). Dry plates 1 days at room temperature, or 30 min with the lids slightly off at 37°C or in a laminar flow hood. Store dried plates wrapped at 4°C.

APPENDIX C HEMACYTOMETER CALCULATION

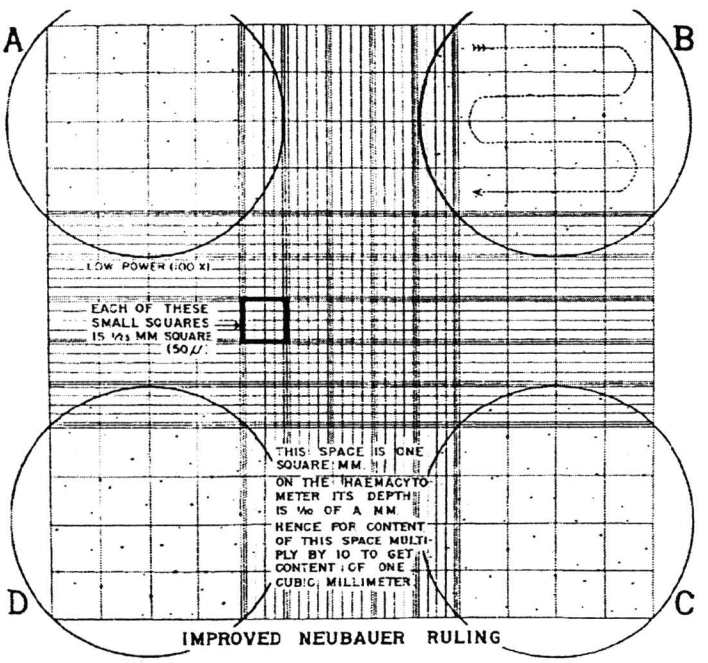

Figure 21 Hemacytometer counting chamber (yeast cells) Areas marked A, B, C, and D are used to count yeast cells

(1) Cover the coglass on to the counting chamber.

(2) Place the forefinger over the top (short end) of the pipet, hold the pipet at a 450 angle, and touch the pipet tip to the junction of the cover glass and the counting chamber.

(3) Allow the yeast suspension to flow under the cover glass until the chamber is completely charged. Similarly, fill the opposite chamber of the hemacytometer.

(4) Count the yeast cells in the four 1 sq mm corner areas corresponding to those marked A, B, C, and D of Figure 25 in each of two chambers.

(5) Count all the yeast cells lying within the square and those touching the upper and right-hand center lines. The yeast cells that touch the left-hand and bottom lines are not to be counted. In each of the four areas, conduct the count as indicated by the "snake-like" line in figure 25. A variation of more than 10 cells between any of the four areas counted or a variation of more than 20 cells between sides of the hemacytometer indicate uneven distribution and require that the procedure be repeated.

Calculation

(1) The depth of the counting chamber is 0.1 mm and the area counted is 4 sq mm (4 squares are counted, each with an area of 1.0 sq mm therefore, 4 x 1.0 sq mm = a total of 4 sq mm). The volume counted is: area x depth = volume. Four sq mm x 0.1 mm = 0.4 cu mm.

(2) The formula is as follows:

$$Yeast\ cells\ (cells/mL) = \frac{Average\ number\ of\ chambers\ (2) yeast\ counted\ x\ dilution\ factor}{Volume\ (0.4)}$$

APPENDIX D

The chemical stability of ethanolic extracts of *A. racemosus.*

The data were expressed as the mean with a standard deviation (SD). Statistical data were analyzed by paired-sample t test. The significance criterion for the correlation measurements was set at 0.05

The statistical chemical stability of ethanolic extracts of *A. racemosus.*

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	AR3E0Day	6.2210	3	.51260	.29595
	AR3E30Day	5.3075	3	.13406	.07740
Pair 2	AR1E0Day	2.0601	3	.25411	.14671
	AR1E30Day	1.6037	3	.64497	.37238

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	AR3E0Day & AR3E30Day	3	-.267	.828
Pair 2	AR1E0Day & AR1E30Day	3	-.972	.151

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	AR3E0Day - AR3E30Day	.91346	.56341	.32528	-.48613	2.31304	2.808	2	.107
Pair 2	AR1E0Day - AR1E30Day	.45638	.89397	.51614	-1.76437	2.67713	.884	2	.470

The calculation of percent remaining of *A. racemosus* extract

The percent remaining of *A. racemosus* extract can be calculated by using the equation;

$$\textbf{Percent remaining} = \frac{C_T}{C_I} \textbf{x 100}$$

where

C_I is the percent saponin equivalent to shatavarin IV of *A. racemosus* extract before stability test.

C_T is the percent saponin equivalent to shatavarin IV of *A. racemosus* extract after stability test.

If initiation, the % saponin equivalent to shatavarin IV of *A. racemosus* extract at normal condition and % saponin of extract at stress condition was 2.06 mg and 1.60 mg, respectively, therefore, the percent remaining of % saponin in *A. racemosus* extract was calculated as follows:

$$\text{Percent remaining} = \frac{1.60}{2.06} \times 100$$

$$= 77.67$$

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Onlom, C., Khanthawong, S., Waranuch, N. and Ingkaninan, K. (2011). Improvement of the bioactivity of *Asparagus racemosus* root extract by saponin-enriched extraction method. In **Proceedings of the Third International Conference on Natural Products for Health and Beauty** (p. 232-236). Bangkok: Rangsit University.

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Petty patent

Ingkaninan, K., Onlom, C., Waranuch, N., Phrompittayarat, W., Khanthawong, S. (2011). **Extraction method for saponin enrichment from *Asparagus racemosus*.** Apply for a Petty Patent, Department of Intellectual Property. (Form PI/PD/PP/001-A) No. 1103000243.