

## APPENDIX A

### YIELD DETERMINATION

Standard is a reference material of known purity and this will be used for the quantification of sample and identification also. Chromatographic peaks of eurycomanone, benzoic acid and gallic acid were identified by referring the retention times of the external standard. External standard method was used to determine the relationship between concentration and area for quantitative interpretation. For this method, the requirements for proper use of external standards are standard solution must containing all elements to be quantified, the standard and the sample matrix should be as similar as possible and analysis conditions for this method must be identical which refers to stable equipment and same sample size.

The concentration for each bioactive compound was determined using standard calibration curve for each bioactive compound based on identified from HPLC chromatogram. The yield of eurycomanone was measured in mg eurycomanone per g of raw material used for extraction. This method was also similar for the yield determination of benzoic acid and gallic acid. Calibration curves were obtained by plotting peak area (y axis,  $\mu\text{V}\cdot\text{sec}$ ) versus the concentrations of external standard (x axis, mg/ml). Millions in the y-axis represents the peak area for HPLC analysis. Standard calibration curve for eurycomanone that was used for determination of eurycomanone concentration, gallic acid and benzoic acid are shown in Figures 1, 2 and 3 respectively.

The response is linear over the entire of concentration range and same injection volume was used for both the unknown (sample) and standard. The results were expressed as the value of correlation coefficient ( $R^2$ ) for eurycomanone, benzoic acid and gallic acid. The following equation represents eurycomanone ( $R^2 = 0.971$ ):

$$y = 3E+07x + 5E+06 \quad (\text{A1})$$

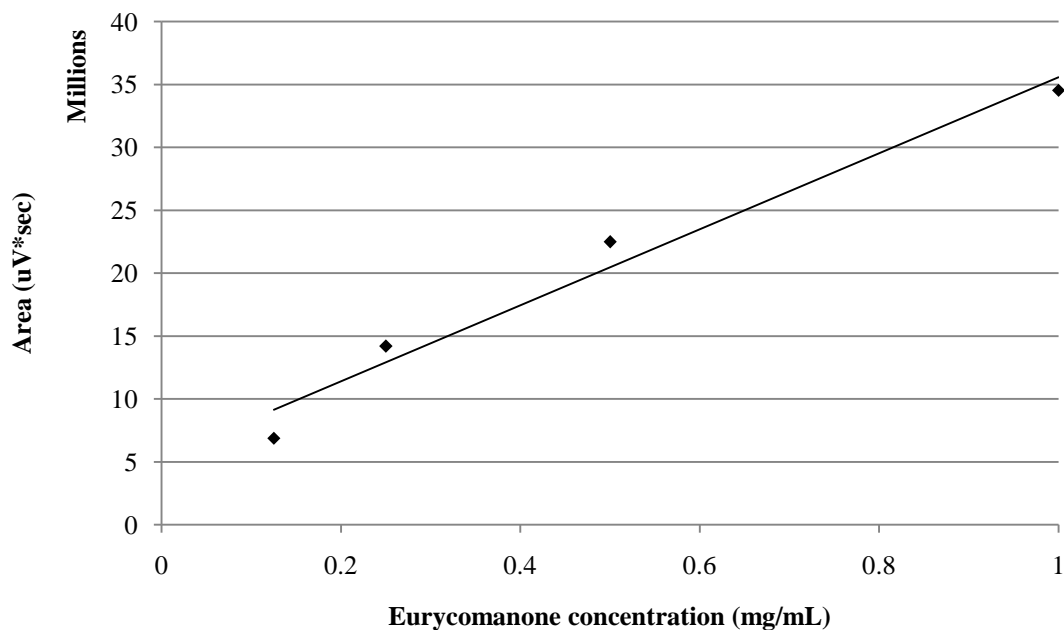
Equation (A2) represents the calibration curve for benzoic acid ( $R^2 = 0.949$ ):

$$y = 5E+06x + 6E+06 \quad (\text{A2})$$

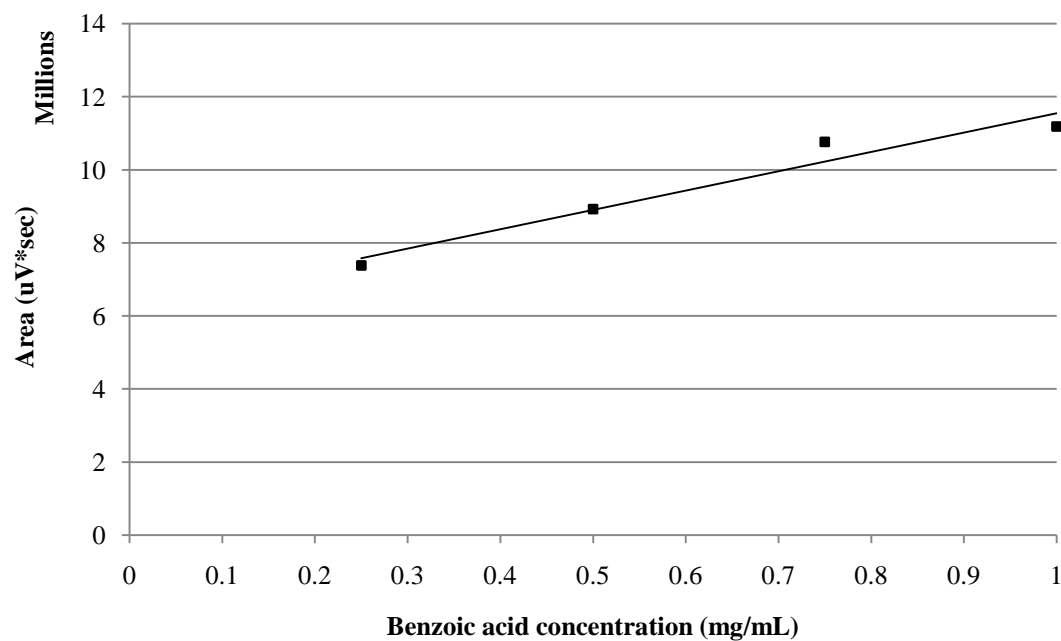
Equation (A3) represents gallic acid ( $R^2 = 0.939$ ):

$$y = 9E+06x + 3E+07 \quad (\text{A3})$$

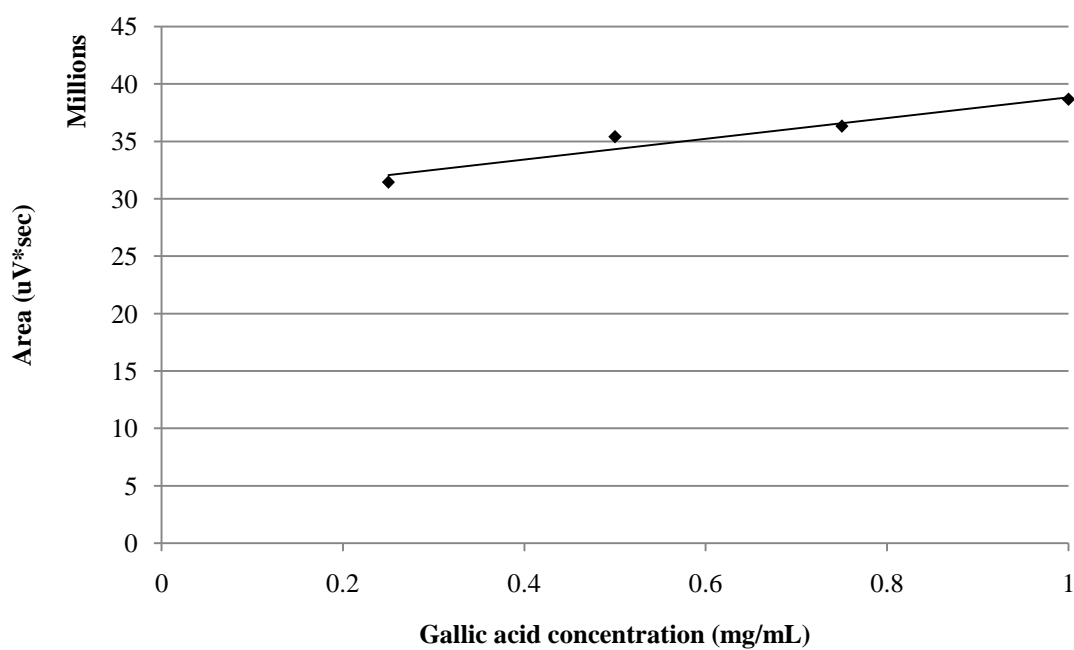
The equations were used for the calculation to determine the concentration of eurycomanone, benzoic acid and gallic acid.



**Figure 1** Standard calibration curve for eurycomanone ( $y = 3E+07x + 5E+06$ ;  $R^2 = 0.971$ )



**Figure 2** Standard calibration curve for benzoic acid ( $y = 5E+06x + 6E+06$ ;  $R^2 = 0.949$ )



**Figure 3** Standard calibration curve for gallic acid ( $y = 9E+06x + 3E+07$ ;  $R^2 = 0.939$ )

The yield of the extraction can be represented in g extract/g raw material or mg eurycomanone/g raw material as calculated in Equation A4. The percentage yield can be calculated by multiplying this equation by 100 %.

$$yield(t) = \frac{C(t)V(t)}{W} \quad (A4)$$

where

$C(t)$  is the concentration of compounds in  $\text{mg}/\text{cm}^3$  at time  $t$ ,

$V(t)$  is the extract volume in  $\text{cm}^3$  at time  $t$ , and

$W$  is the raw sample weight in g.