

## REFERENCES

1. L. Buricova and Z. Reblova, *Czech Journal of Food Sciences*, 26 (2008) 132-138
2. J. Jakmunee and K. Grudpan, *Analytica Chimica Acta*, 438 (2001) 299–304
3. I.F.F. Benzie and J.J. Strain, *Analytical Biochemistry*, 239 (1996) 70-76
4. R. Apak, K. Guclu, M. Ozyurek and S.E. Karademir, *Journal of Agricultural and Food Chemistry*, 52 (2004) 7970-7981
5. J. Jakmunee, *Handbook of electroanalysis – 203732*, Department of chemistry, Faculty of Science, Chiang Mai university, 2006
6. K. Shetty, G. Paliyath, A. Pometto, and R. E. Levin, *Food Biotechnology*, Second Edition, CRC Press, Florida, U.S., 2006
7. <http://en.wikipedia.org/wiki/Antioxidant> (accessed on 7 June 2011)
8. M. Seruga, I. Novak, L. Jakobek, *Food Chemistry*, 124 (2011) 1208-1216
9. A. Rodrigue-Bernaldo de Quiros, M.A. Lage-Yusty, J. Lopez-Hernandez, *Food Research International*, 42 (2008) 1018-1022
10. B. Saad, Y. Yek Sing, M. Asri Nawi, N. Hasain Hashim, A. Salhin Mohamed Ali, M. Idiris Saleh, S. Fariza Sulaiman, K. Md Talib, K. Ahmad, *Food Chemistry*, 105 (2007) 289-394
11. E. Fries, W. Puttmann, *Water Research*, 36 (2002) 2319-2327
12. M.R. Lee, C.Y. Lin, Z.G. Li and T.F. Tsai, *Journal of Chromatography A*, 1120 (2006) 244-251
13. J. Cheng and P. Jandik, *Journal of Chromatography A*, 1198 (2008) 148-153

14. K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D. Hawkins Byrne, *Journal of Food Composition and Analysis*, 19 (2006) 669-675
15. A. Floegel, D.O. Kim, S.J. Chung, S.I. Koo, O.K. Chun, *Journal of Food Composition and Analysis*, 24 (2011) 1043-1048
16. J. Lachman, K. Hamouz, M. Orsak, V. Pivec and P. Dvorak, *Science Horticulture*, 117 (2008) 109-114
17. T. Mezdari, D. Villano, M.S. Fernandez-Pachon, M.C. Garcia-Parrilla and A.M. Troncoso, *Journal of Food Composition and Analysis*, 21 (2008) 282-290
18. P.C. Wootton-Beard, A. Moran and L. Ryan, *Food Research International*, 44 (2011) 217-224
19. F. Biglari, A.F.M Alkarkhi and A.M. Easa, *Food Chemistry*, 107 (2008) 1636-1641
20. C.C. Teow, V.D. Troung, R.F. McFeeters, R.L. Thompson, K.V. Pecota and G.C. Yencho, *Food Chemistry*, 103 (2007) 829-839
21. N. Turkmen, F. Sari and Y. S. Velioglu, *Food Chemistry*, 99 (2006) 835-841
22. N. Cicco, M.T. Lanorte, M. Paraggio, M. Viggiano and V. Lattanzio, *Microchemical Journal*, 91 (2009) 107-110
23. D. Ozyurt, B. Demirata and R. Apak, *Talanta*, 71 (2007) 1155-1165
24. P. Siddhuraju, *Food Chemistry*, 99 (2006) 19-157
25. S.C. Langley-Evans, *International Journal of Food Science and Nutrition*, 51 (2000) 181-188
26. E. Bursal and E. Koksak, *Food Research International*, 44 (2011) 2217-2221
27. H. Li, X. Wang, Y. Li, P. Li and H. Wang, *Food Chemistry*, 112 (2009) 454-460

28. I.F. Abdullin, E.N. Turova, G.K. Ziyatdinova and G.K. Budnikov, *Journal of Analytical Chemistry*, 57 (2002) 353-355
29. S. Milardovic, D. Ivekovic and B.S. Grabaric, *Bioelectrochemistry*, 68 (2006) 175-180
30. H. Alemu, B.M. Abegaz and M. Bezabih, *Bulletin of the Chemical Society of Ethiopia*, 21 (2007) 189-204
31. R.A. Medeiros, R.C. Rocha-Filho and O. Fatibello-Filho, *Food Chemistry*, 123 (2010) 886-891
32. A.M. Pisoschi, M.C. Cheregi and A.F. Danet, *Molecules*, 14 (2009) 480-493
33. L.K. Shpigun, M.A. Arharova, K.Z. Brainina and A.V. Ivanova, *Analytical Chimica Acta*, 573-574 (2006) 419-426
34. S. Milardovic, I. Kerekovic and V. Rumenjak, *Food Chemistry*, 105 (2007) 1688-1694
35. S. Milardovic, D. Ivekovic and B.S. Grabaric, *Bioelectrochemistry*, 68 (2006) 175-180
36. S. Mannino, S. Buratti, M.S. Cosio and N. Pellegrini, *Analyst*, 124 (1999) 1115-1118
37. R.A. Medeiros, B.C. Lourencao, R.C. Rocha-Filho and O. Fatibello-Filho, *Analytical Chemistry*, 82 (2010) 8658-8663
38. Y.T. Hung, P.C. Chen, R.L.C. Chen and T.J. Cheng, *Sensors and Actuators B*, 130 (2008) 135-140
39. S. Buratti, M. Scampicchio, G. Giovanelli and S. Mannino, *Talanta*, 75 (2008) 312-316
40. A.M. Pisoschi, M.C. Cheregi and A.F. Danet, *Molecules*, 14 (2009) 480-493

41. V.A. Pedrosa, A.R. Malagutti, L.H. Mazo and L. A. Avaca, *Analytical Letters*, 39 (2006) 2737–2748
42. J. Ruzicka, *Flow injection analysis ebook*, 2<sup>nd</sup> ed., FIALab Instruments Inc., Washington, USA 2002
43. F.A. Settle, *Handbook of instrumental techniques for analytical chemistry*, Prentice Hall PTR, New Jersey, USA ,1997
44. [http://en.wikipedia.org/wiki/Ascorbic\\_acid](http://en.wikipedia.org/wiki/Ascorbic_acid) (accessed on 14 February 2011)
45. <http://en.wikipedia.org/wiki/Trolox> (accessed on 14 February 2011)
46. <http://chemistry.about.com/od/factsstructures/ig/Chemical-Structures---T/Alpha-Tocopherol.htm> (accessed on 14 February 2011)
47. [http://www.ganfyd.org/index.php?title=Alpha\\_tocopheryl\\_acetate](http://www.ganfyd.org/index.php?title=Alpha_tocopheryl_acetate) (accessed on 14 February 2011)
48. [http://en.wikipedia.org/wiki/Gallic\\_acid](http://en.wikipedia.org/wiki/Gallic_acid) (accessed on 14 February 2011)
49. <http://www.vcharkarn.com/vcafe/42064> (accessed on 14 February 2011)
50. [http://www.rdchemicals.com/chemicals.php?mode=details&mol\\_id=7570](http://www.rdchemicals.com/chemicals.php?mode=details&mol_id=7570) (accessed on 14 February 2011)
51. <http://en.wikipedia.org/wiki/Quercetin> (accessed on 14 February 2011)
52. <http://en.wikipedia.org/wiki/Catechin> (accessed on 14 February 2011)
53. <http://en.wikipedia.org/wiki/File:Epicatechin.png> (accessed on 14 February 2011)
54. <http://www.agrool.gr/files/p-coumaric%20acid.jpg> (accessed on 14 February 2011)
55. [http://en.wikipedia.org/wiki/File:Caftaric\\_acid.svg](http://en.wikipedia.org/wiki/File:Caftaric_acid.svg) (accessed on 14 February 2011)

56. [http://www.rdchemicals.com/chemicals.php?mode=details&mol\\_id=8170](http://www.rdchemicals.com/chemicals.php?mode=details&mol_id=8170)  
(accessed on 14 February 2011)
57. <http://en.wikipedia.org/wiki/Rutin> (accessed on 14 February 2011)
58. <http://classifieds.agriscape.com/ad/161776/en/> (accessed on 14 February 2011)
59. <http://en.wikipedia.org/wiki/File:Kaempferol.png> (accessed on 14 February 2011)
60. <http://en.wikipedia.org/wiki/File:Myricetin.png> (accessed on 14 February 2011)
61. [http://2008.igem.org/Team:Rice\\_University/BACKGROUND](http://2008.igem.org/Team:Rice_University/BACKGROUND) (accessed on 14 February 2011)
62. [http://en.wikipedia.org/wiki/Propyl\\_gallate](http://en.wikipedia.org/wiki/Propyl_gallate) (accessed on 14 February 2011)
63. [http://www.ask.com/wiki/Butylated\\_hydroxyanisole](http://www.ask.com/wiki/Butylated_hydroxyanisole) (accessed on 14 February 2011)
64. <http://www.made-in-china.com/showroom/ghy20000/product-detailBfuQJMrsxok/China-Butylated-Hydroxytoluene-BHT-.html> (accessed on 14 February 2011)
65. <http://en.wikipedia.org/wiki/Tert-Butylhydroquinone> (accessed on 14 February 2011)
66. <http://www.etholttc.com/consultancy-en-US/natural-products/> (accessed on 14 February 2011)

## **APPENDICES**

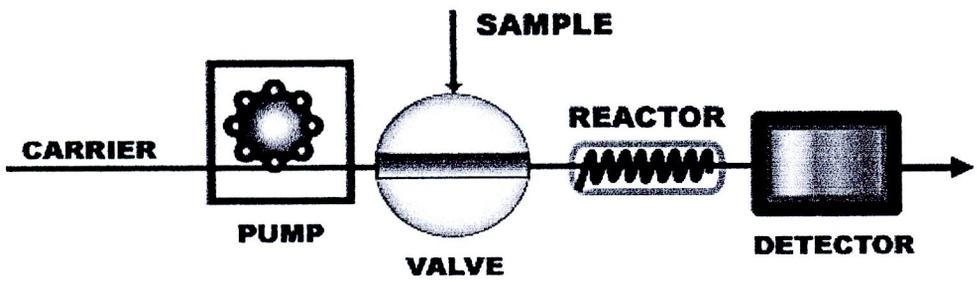
## APPENDIX A

### Flow Injection analysis

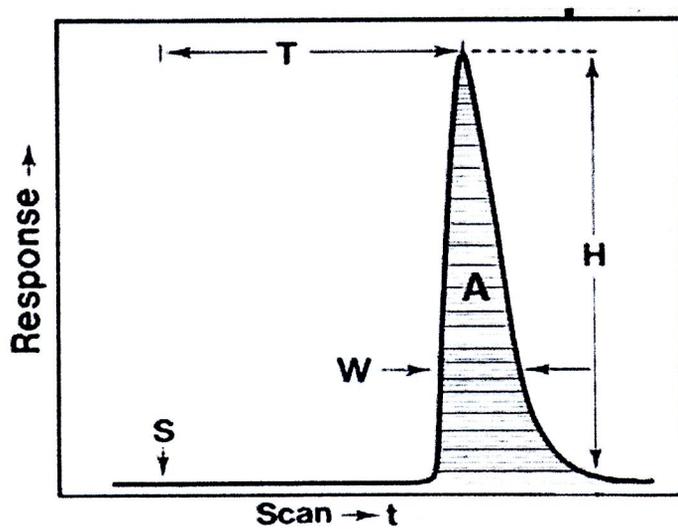
Flow injection analysis (FIA) is based on the injection of a liquid sample into a moving continuous carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a flow through detector that continuously records as a signal such absorbance, potential or other physical parameters.

Three principle of FIA are sample injection, controlled dispersion of the injected sample zone and reproducible timing of its movement from the injection point toward and into the detector. This permits obtaining a highly reproducible readout even when the mixing is incomplete, the chemistry may not reach equilibrium and the signal is transient [42].

The simplest flow injection analyzer is shown in Figure 1(a). That consists of a pump which is used to propel the carrier stream through a narrow tube. An injection port where a well-defined volume of a sample solution is injected into the carrier stream in a reproducible manner. A reactor or mixing coil is where the sample zone disperses and reacts with the components of the carrier stream, forming a species that is sensed by a flow-through detector and recorder. A typical recorder output has the form of a peak. The height and area of peak are generally evaluated their values are related to the concentration of the analyte. The shape of peak is mainly dependent on the physical process of dispersion of sample zone and the chemical process of the formation of the products, which occur at the same time [42].



(a)



(b)

**Figure 1** The simplest flow injection analyzer (a) and the FI-response (b) [42].

## **APPENDIX B**

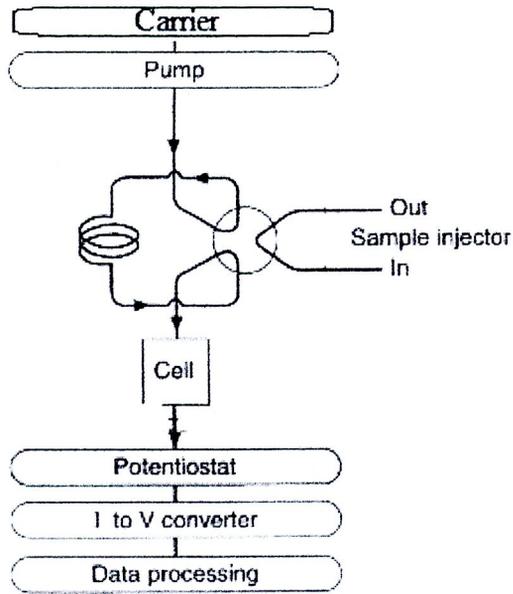
### **Amperometric method**

#### **1. Principle of amperometric method**

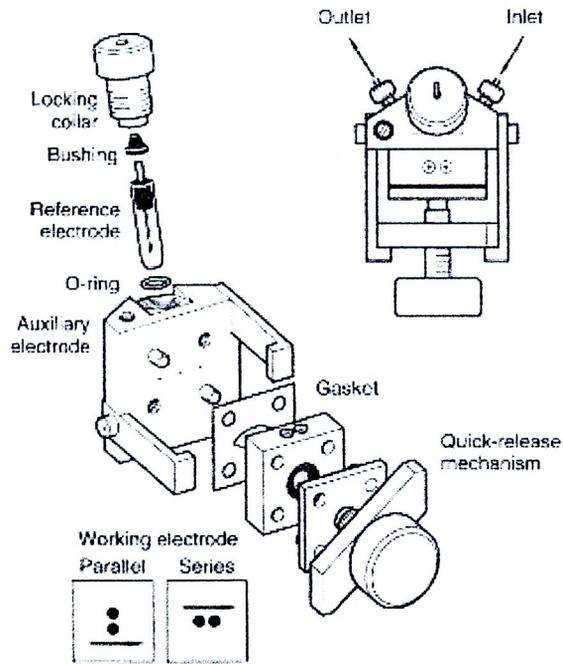
Amperometric method is based on the measurement of current at a fixed potential. Either an oxidation or reduction is forced to occur by judicious selection of the potential applied to an electrode by a controlling potentiostat. The electrode acts as an oxidizing or reducing agent of variable power. In order to use amperometric measurements effectively, it is important to recognize that electrochemical detection is a surface technique, which means molecules not adjacent to the electrode must be moved to the surface to react. Flow injection analysis has been combined with amperometric detection. The various electrodes are placed in a flowing stream configured as a thin film. The film thickness is variable, with typical values being 15 – 125  $\mu\text{m}$  [43]. The potential applied to the electrochemical cell between the reference and working electrode serves as the driving force for the detection redox reaction to occur. The working potential is usually determined by producing a current-potential plot. This is accomplished by monitoring the current at various potentials. At low positive (negative) potentials, the energy applied to the cell is insufficient. As the applied potential increases in the positive (negative) direction, the energy requirement for the reaction is now partially met, and a faradaic current ensues. As the potential is further increased, the faradaic current rises until a potential is reached, past which no further improvement in the current response is noted [43].

## 2. Instrumentation of flow injection amperometric system

The basic components of such a flow injection amperometric system is shown in Figure 1, which consist of a pump that provides a constant flow of carrier stream to an injection valve where the sample is introduced. The requirement of carrier stream must have low electrochemical activity that is low background current. A sample injection port may be manual or automated by should be of short durations. An injected sample may pass through a tube directly to the amperometric cell. A transducer cell is illustration in Figure 2. The thin-layer channel is defined by a gasket held between a stainless-steel block and a polymeric block is also. The stainless-steel block is the auxiliary electrode and provides a compartment for the reference electrode. The polymeric block contains the working electrodes. The potential selected by the user is applied between the reference and working electrodes while the current is passed between the auxiliary and working electrodes. Detection occurs at the working electrode in the thin-layer region. Electrodes of the same or different materials may be interchanged by simply swapping the working electrode half of the thin-layer cell. Glassy carbon and carbon paste have been used. This design allows easy collection of solute band without appreciable dispersion. Electrodes must have diameters of a few millimeters. Figure 2 also schematically depicts a thin – layer cell with two working electrodes, which can be arranged in a series or parallel configuration relative to a flow stream. A different potential can be applied to each electrode and the current of each monitored [43]



**Figure 1** The basic components of flow injection amperometric system [43]

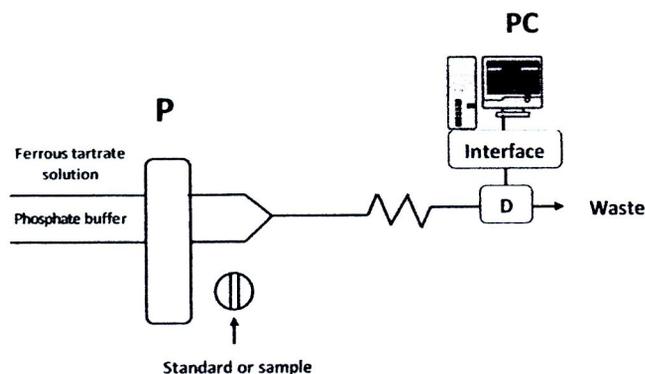


**Figure 2** The amperometric cell [43].

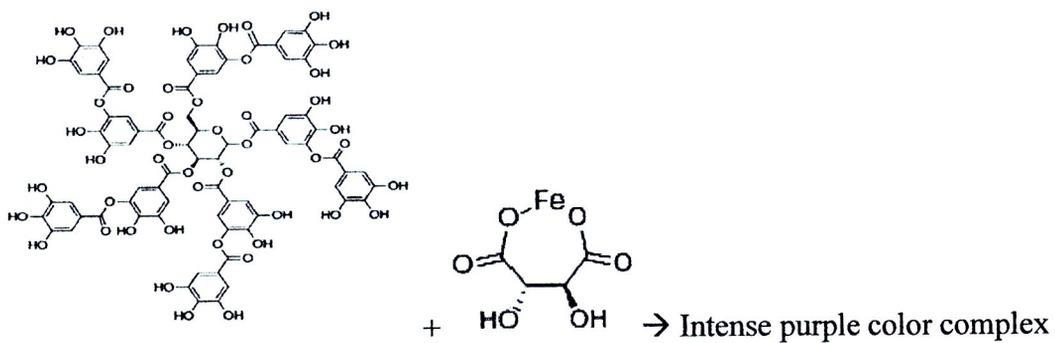
## APPENDIX C

### FI – ferrous tartrate method

FI – ferrous tartrate method for estimation of antioxidant activity is shown in Figure 1. The system is comprised of peristaltic pump (Ismatec, Switzerland) with pump tubing, six port injection valve (Upchurch Scientific, USA), a mixing coil (PTFE, i.d. 0.5 mm), and a homemade flow through colorimeter. Sample or standard solution was injected into the carrier stream and mixed in-line with the reagent solution. The antioxidants react with ferrous tartrate solution leading to an increase in the absorbance of complex which could be detected by a flow through colorimeter at 540 nm and was the signal recorded as FIA peak. The reaction involved is shown in scheme 1. Antioxidative activity (as tannin equivalent) was calculated from a calibration equation, plotting between the peak height obtained and concentration of tannic acid.



**Figure 1** Manifold for estimation of antioxidant activity by FI – ferrous tartrate method. P = Pump, D = homemade flow through colorimeter, PC = Computer

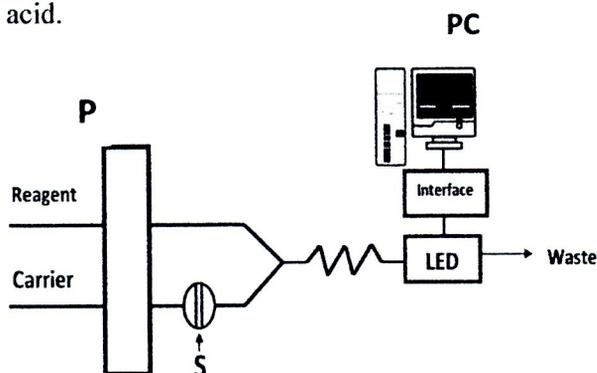


**Scheme 1** Reaction between ferrous tartrate and tannin [21]

## APPENDIX D

### FI – colorimetric method based on FRAP reaction

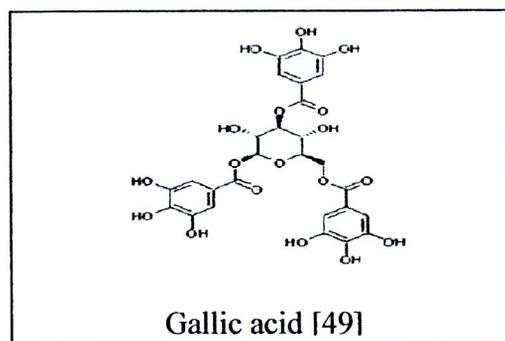
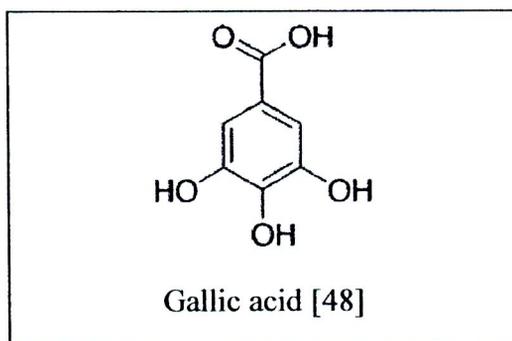
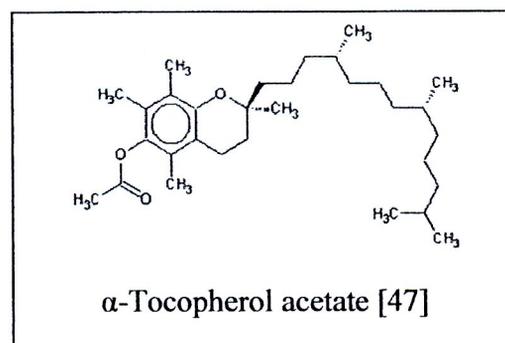
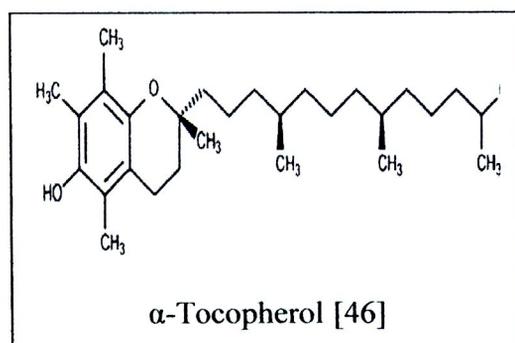
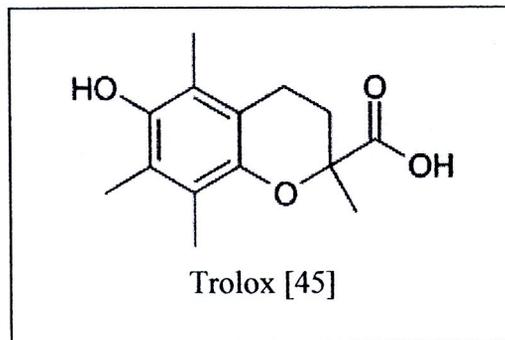
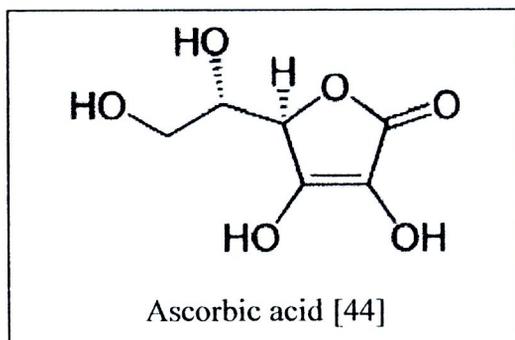
FI – colorimetric method based on FRAP reaction for estimation of antioxidant activity is shown in Figure 1. The system consists of a peristaltic pump (Ismatec, Switzerland) with pump tubing, a six-port injection valve (Ogawa, Japan) with injection loop 50  $\mu\text{L}$ , a mixing coil (PTFE, i.d. 0.5 mm) and a homemade flow through colorimeter. The system was controlled by a personal computer using a software program written in-house. Sample or standard solution was injected into the carrier stream and mixed in-line with the reagent solution. The antioxidants react with Fe(III) to produce Fe(II) leading to an increase in the absorbance of Fe(II) - 1,10-phenanthroline complex which could be detected by a colorimeter with green LED as light source and the signal was recorded as FIA peak. Antioxidative activity (as ascorbic acid equivalent) was calculated from a calibration equation, plotting between the peak height obtained and concentration of ascorbic acid.

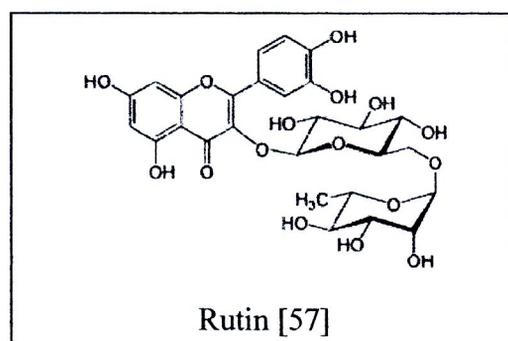
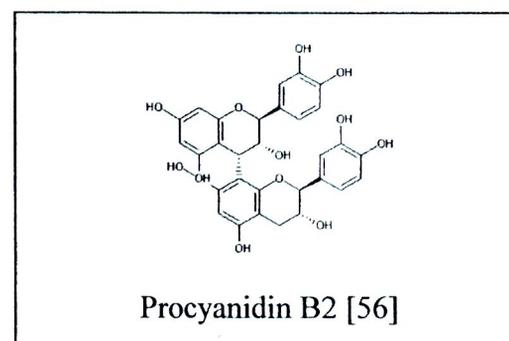
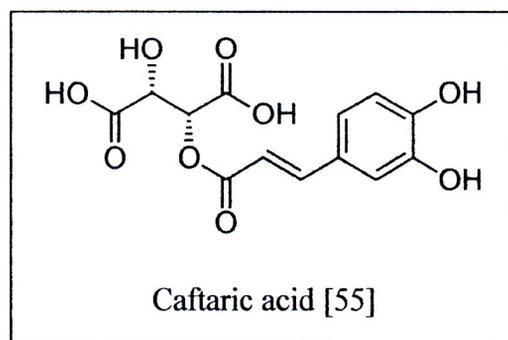
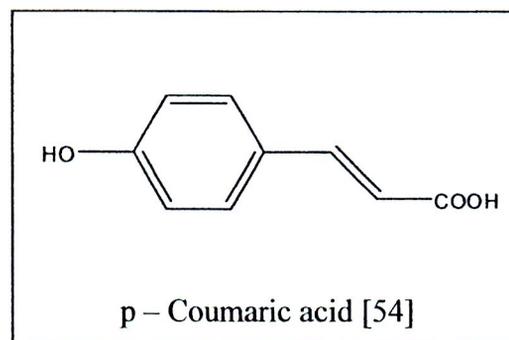
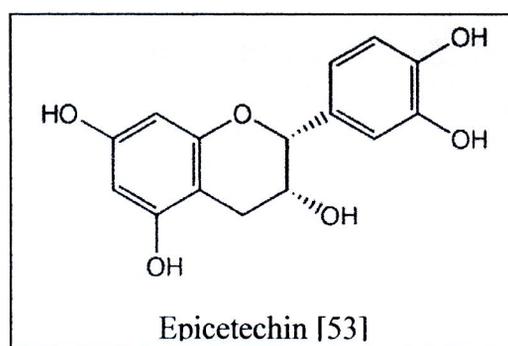
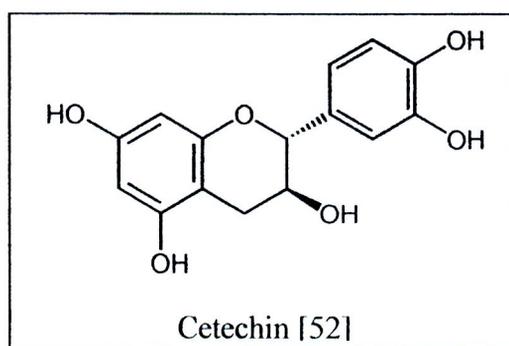
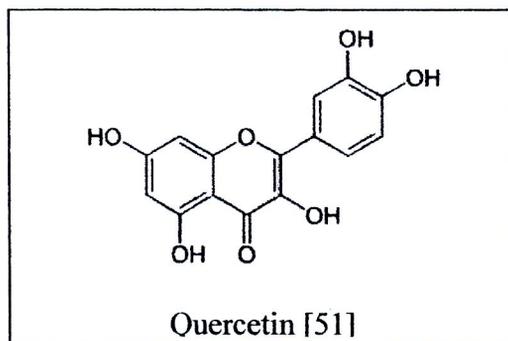
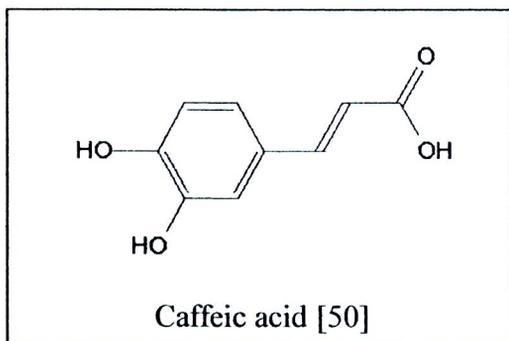


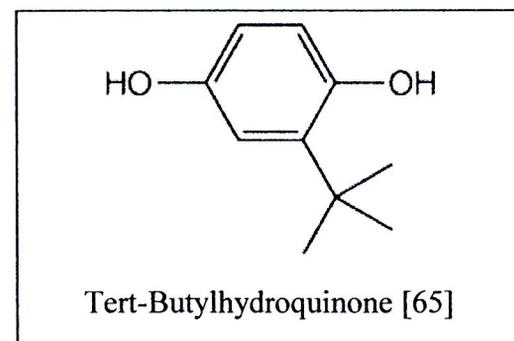
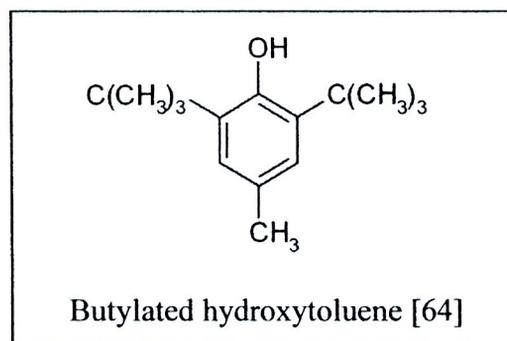
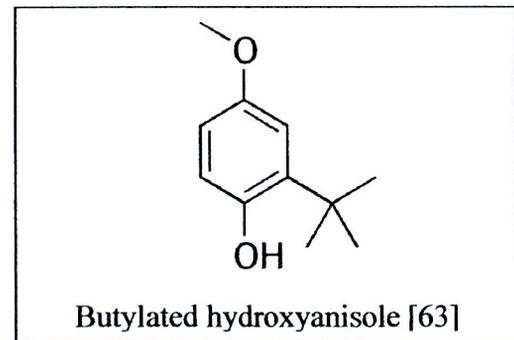
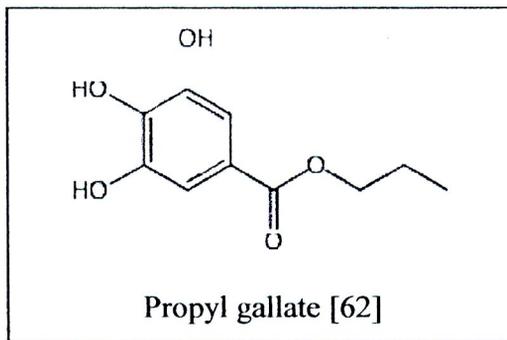
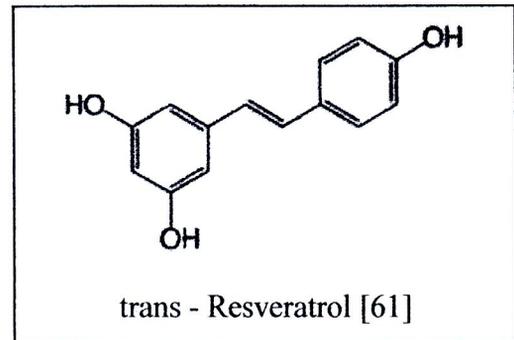
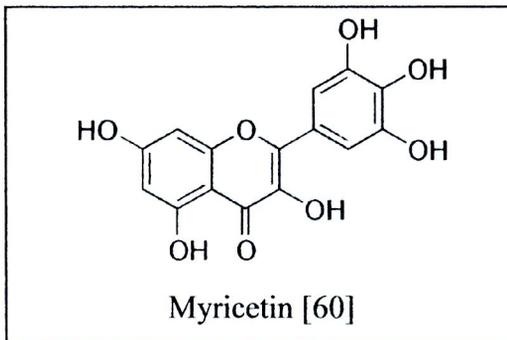
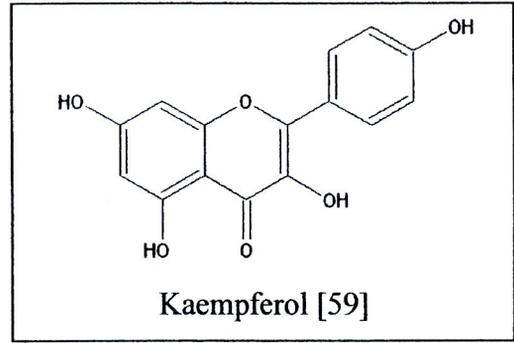
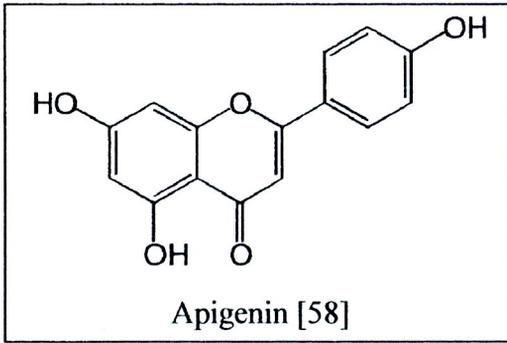
**Figure 1.** Manifold of flow injection colorimetric method based on FRAP reaction for estimation of antioxidative activity. S = standard/sample, Carrier = Deionized water and Reagent = 1.5 mM Fe(III) – 1.5 mM 1,10-phenanthroline.

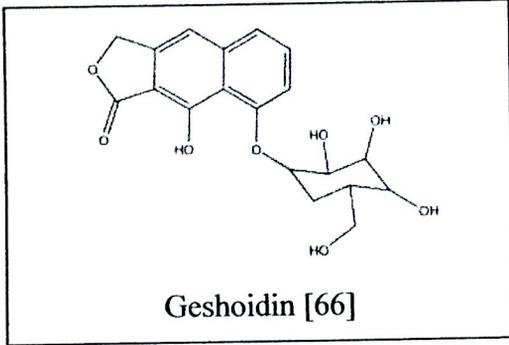
## APPENDIX E

### Structure of some antioxidant compounds









## **THE RELEVANCY OF THE RESEARCH WORK TO THAILAND**

Estimation of antioxidant becomes attractive in Thailand because antioxidants can prevent many human diseases which are affected by free radicals. Antioxidant contains in various types of sample such as tea, vegetable, fruit and herb. So the estimation of antioxidant activity in natural sample is necessary for using these samples as ingredients in dietary supplements and cosmetic or pharmaceutical. Many commercial analytical methods such as chromatography and spectrophotometry are employed for analysis of antioxidant activity. However most of these methods rely on sophisticated and expensive instruments and consume long analysis time that makes their uses quite limited in specific groups.

This research aims to develop the FI amperometric method for estimation of antioxidant and applied the method to the analysis of real sample such as green teas and Thai herb infusion tea. The proposed method provided high a satisfactory sample throughput. By taking advantages of FI method, not only low consumption the amount of reagent and sample but the analysis time also less than those of the commonly used instrument that reduces cost of operation. The analytical performance was comparable to the traditional method which should be beneficial to apply in small manufactories or even in large sector in Thailand.

## **CURRICULUM VITAE**

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**Date of birth** March 29, 1988

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2009 B.S. (Chemistry), Chiang Mai University

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2010-2011 Full support from Human Resource Development in Science

### **Experience**

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- 2011 Graduate Teaching Assistant Electroanalysis Laboratory course (203732) at Department of Chemistry, Faculty of Science, Chiang Mai University
- 2011 Committee assistant in a workshop on “New technology for determination of plant nutrients in soil and fertilizers for properly management”, Department of Chemistry, Faculty of Science, Chiang Mai University, 20 May 2011
- 2011 Committee assistant in a workshop on “New method of analysis of heavy metals in ceramic products by anodic stripping voltammetry”, Department of Chemistry, Faculty of Science, Chiang Mai University, 22 July 2011
- 2011 Committee assistant for 84th RGJ Seminar Series: Research and Innovation in Chemistry for Sustainable Development, University Academic Service Center (UNISERV), Chiang Mai University, Thailand, 2 September 2011.

## **Publication**

### **International conferences**

1. Puttaporn Klayprasert and Jaroon Jakmune, “Flow injection Amperometric Detection for Screening of Antioxidative Activity” (Poster Presentation), The 2<sup>nd</sup> Regional Electrochemistry Meeting of South-East Asia on Applied

Electrochemistry for Modern Life (2<sup>nd</sup> REMSEA 2010), Maha Chulalongkorn Building, Chulalongkorn University, Thailand, 16-19 November 2010.

2. Puttaporn Klayprasert, Nareerat Hemloha, Porntip Rakshasawad and Jaroon Jakmune, “Flow Injection Colorimetric Method base on FRAP Reaction for Estimation of Antioxidative Activity” (Poster Presentation), Pure and Applied Chemistry International Conference (PACCON2012), The Empress Hotel, Chiang Mai, Thailand, 11-13 January 2012.

3. Puttaporn Klayprasert and Jaroon Jakmune, “Development of Flow Injection Amperometric Method Using Triiodide Ion as Reagent for Estimation of Antioxidant Activity” (Oral presentation), Pure and Applied Chemistry International Conference (PACCON2012), The Empress Hotel, Chiang Mai, Thailand, 11-13 January 2012.

### **National conference**

1. Puttaporn Klayprasert and Jaroon Jakmune, “Flow Injection Amperometry for Determination of Total Antioxidant” (Poster Presentation), The 6<sup>th</sup> Conference on Science and Technology for Youth 2011, Bangkok International Trade and Exhibition Centre, Thailand, 18-19 March 2011.



