APPENDIX

A. GENERAL METHODS

A-1 Preparation of Mueller Hinton agar slant

A 100 mL agar medium comprising of 3.8 g Mueller Hinton agar and 0.5 g of agar powder by heating in boiling distilled water to dissolve completely. The medium (approximate 5 mL) was poured into test tubes before sterilization at 121 ◦C for 15 min. Then the sterile agar medium was solidified slantingly laying.

A-2 Preparation of potato dextrose agar slant

A 100 mL agar medium comprising of 3.9 g Potato dextrose agar and 0.5 g agar powder by heating in boiling distilled water to dissolve completely. The medium (approximate 5 mL) was poured into test tubes before sterilization at 121 ◦C for 15 min. Then the sterile agar medium was solidified slantingly laying.

A-3 Preparation of Mueller Hinton agar plate

Muller Hinton agar (3.8 g) and agar powder (0.5 g) were melted by heating in 100 mL of boiling distilled water, after that sterilized at 121 ◦C for 15 min. Then, twenty-five mL of the sterile medium were poured into each Petri dish.

A-4 Preparation of Potato dextrose agar plate

Potato dextrose agar (3.9 g) and agar powder (0.5 g) were melted by heating in 100 mL of boiling distilled water, after that sterilized at 121 °C for 15 min. Then, twenty-five mL of the sterile medium were poured into each Petri dish.

A-5 Preparation of Mueller Hinton broth

Mueller Hinton broth (2.1 g) was melted by heating in 100 mL of boiling distilled water, after that sterilized at 121 °C for 15 min.

A-6 Preparation of Potato dextrose broth

Potato dextrose agar (3.9 g) was melted by heating in 100 mL of boiling distilled water, after that sterilized at 121 °C for 15 min.

A-7 Preparation of cell suspension

A few colonies of bacteria were aseptically transferred into screw-capped vials containing sterile distilled water. The turbidity of the cell suspensions was then adjusted with the sterile distilled water to obtain turbidity visually comparable to that of a turbidity standard prepared by adding 0.5 mL of 1.175% w/v barium chloride dehydrate ($BaCl_2 \cdot 2H_2O$) solution to 99.5 mL of 1% v/v sulfuric acid (H_2SO_4). This turbidity is the density of a McFarland 0.5 standard.

A-8 Preparation of fungal spore suspension

Spores from the cultures grown in potato dextrose agar slants were aseptically transferred into screw-capped vials containing sterile distilled water. They were vigorously shaken and then adjusted with the sterile distilled water to obtain turbidity visually comparable to that of a turbidity of McFarland 0.5 standard.

A-9 Preparation of chitosan solution

Chitosan 1% w/v was prepared by dissolving 1 g of chitosan powder (92% deacetylation) in 100 mL of 0.5% v/v acetic acid acetic acid in distilled water, with agitation overnight before sterilization at 121 ˚C for 15 min.

A-10 Preparation of phosphate buffer (0.04 M, pH 7.0)

Potassium dihydrogenphosphate (KH_2PO_4) 3.361 g and potassium hydrogenphosphate (K_2HPO_4) 2.646 g were dissolved in 800 mL of distilled water and adjusted to pH 7.0 and then topped off to 1000 mL with distilled water.

A-11 Preparation of ammonium thiocyanate solution (30% w/v)

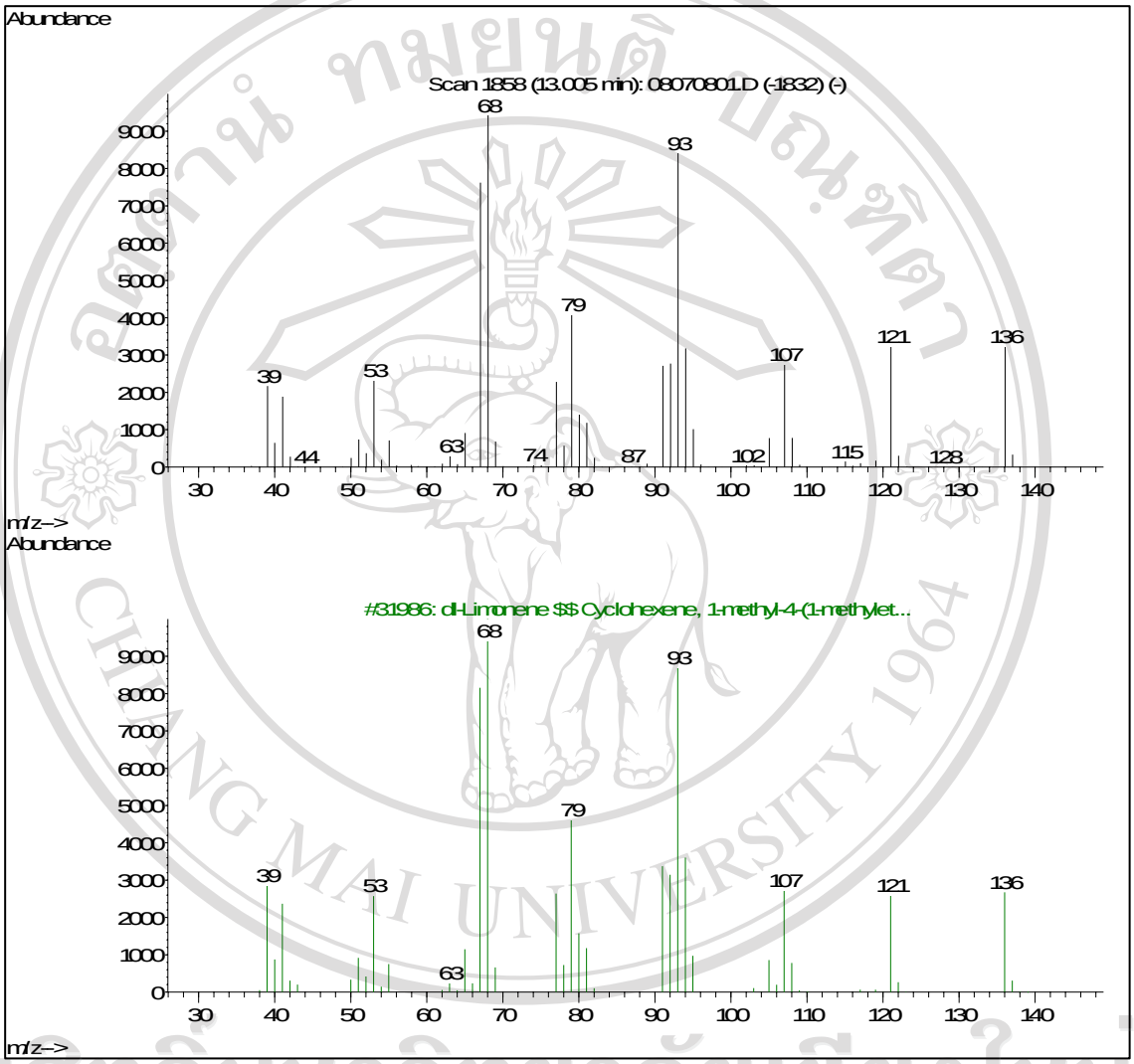
Ammonium thiocyanate 7.50 g was dissolved in distilled water. This solution was mixed well and adjusted to 25.00 mL with distilled water.

A-12 Preparation of ferrous chloride solution (20 mM in 3.5% HCl)

Hydrochloric acid (HCl) 3.5% was prepared by pipette 2.4 mL of conc. HCl into 25 mL volumetric flask containing water and then topped off to 25 mL with water. A 0.1 g of ferrous chloride was dissolved with 25 mL of 3.5% HCl.

B. Mass spectrum of the essential oil fraction and the structure of main components

a)



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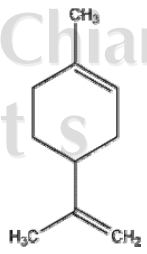
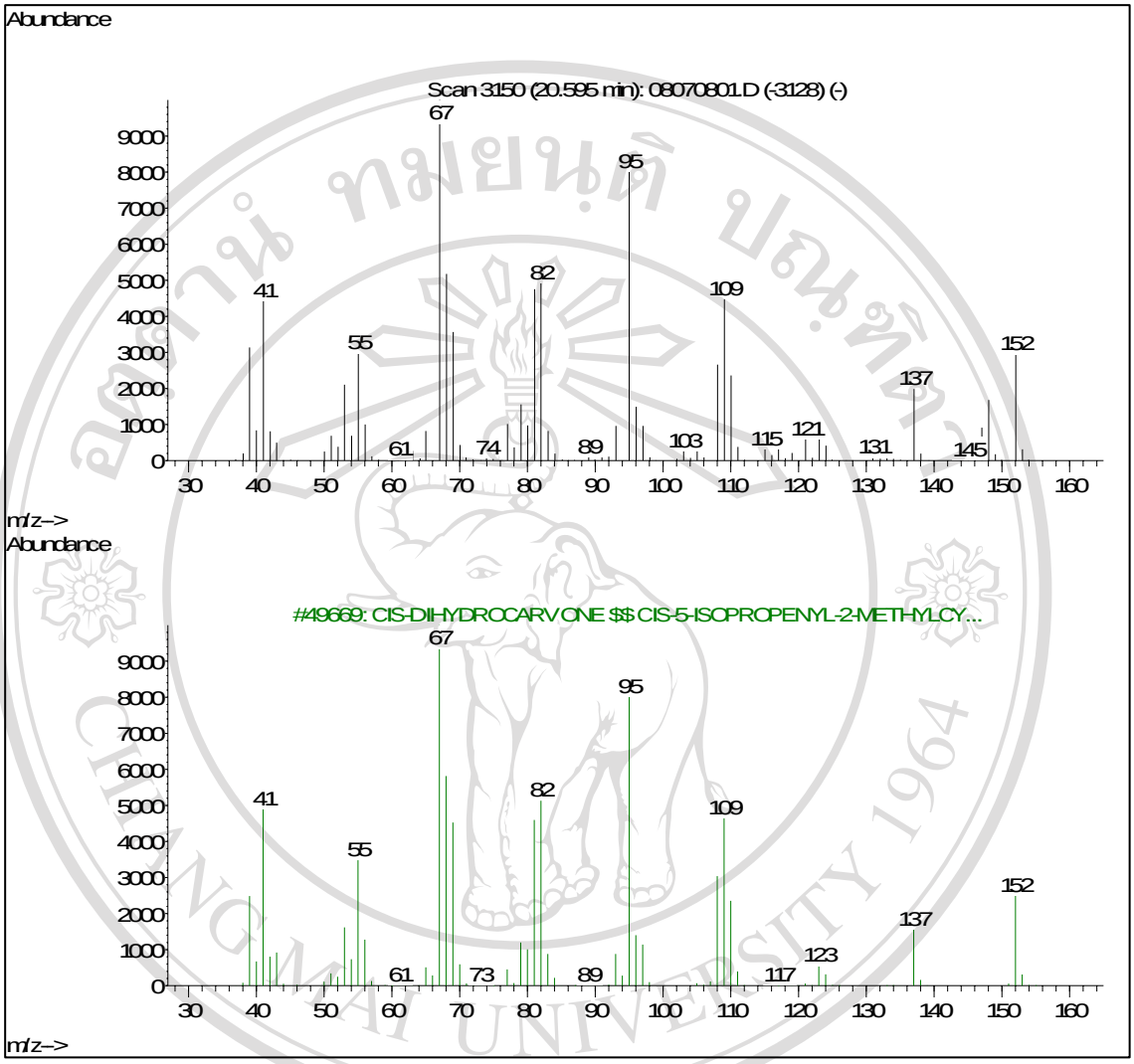


B-1 a) Mass spectrum of *A. graveolens* at retention time 13.005 min (above), and reference spectrum of dl-limonene (bottom) b) Structure of dl-limonene

a)



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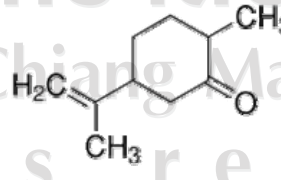
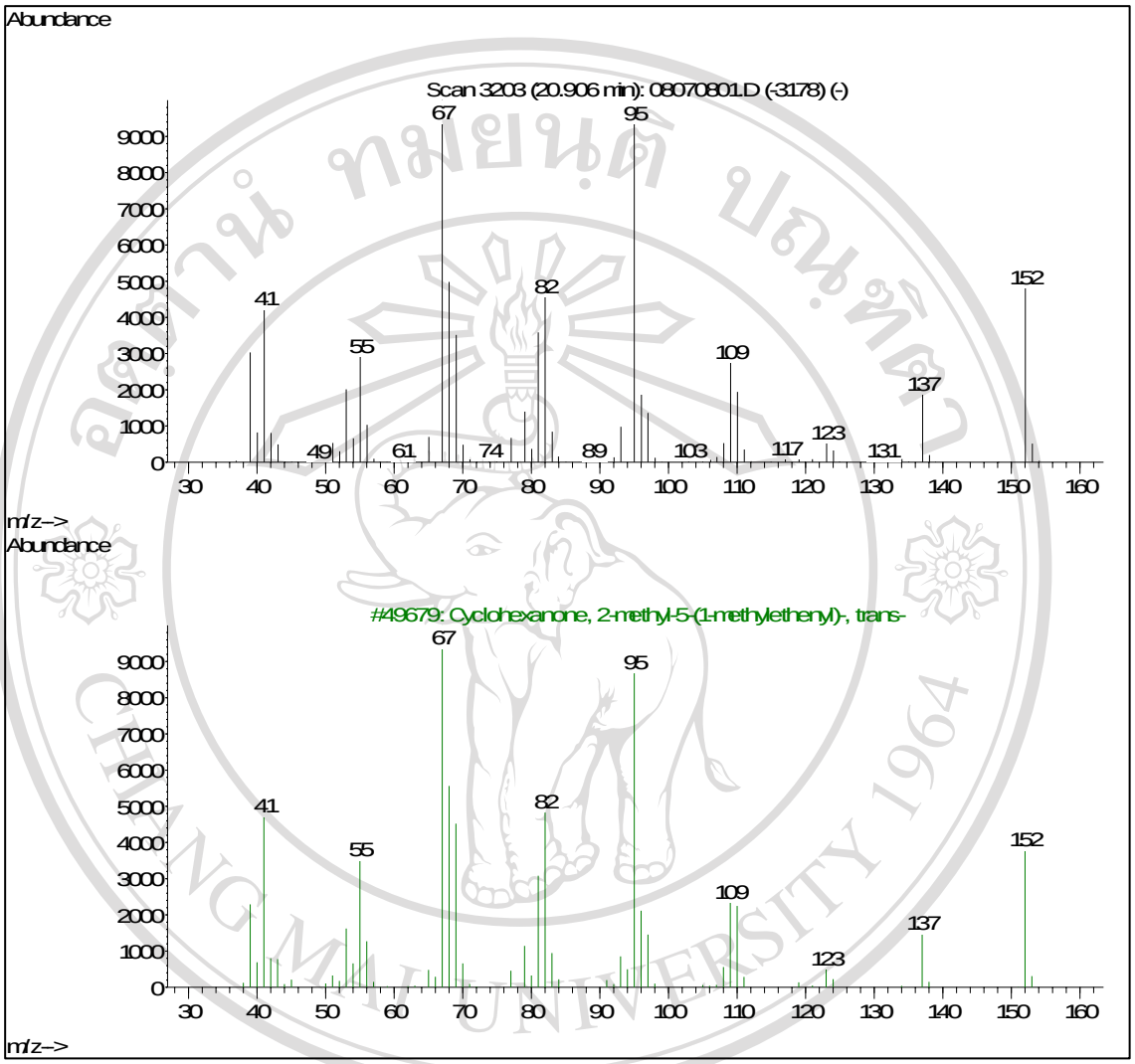


B-2 a) Mass spectrum of *A. graveolens* at retention time 20.595 min (above), and reference spectrum of cis-dihydrocarvone (bottom) b) Structure of cis-dihydrocarvone

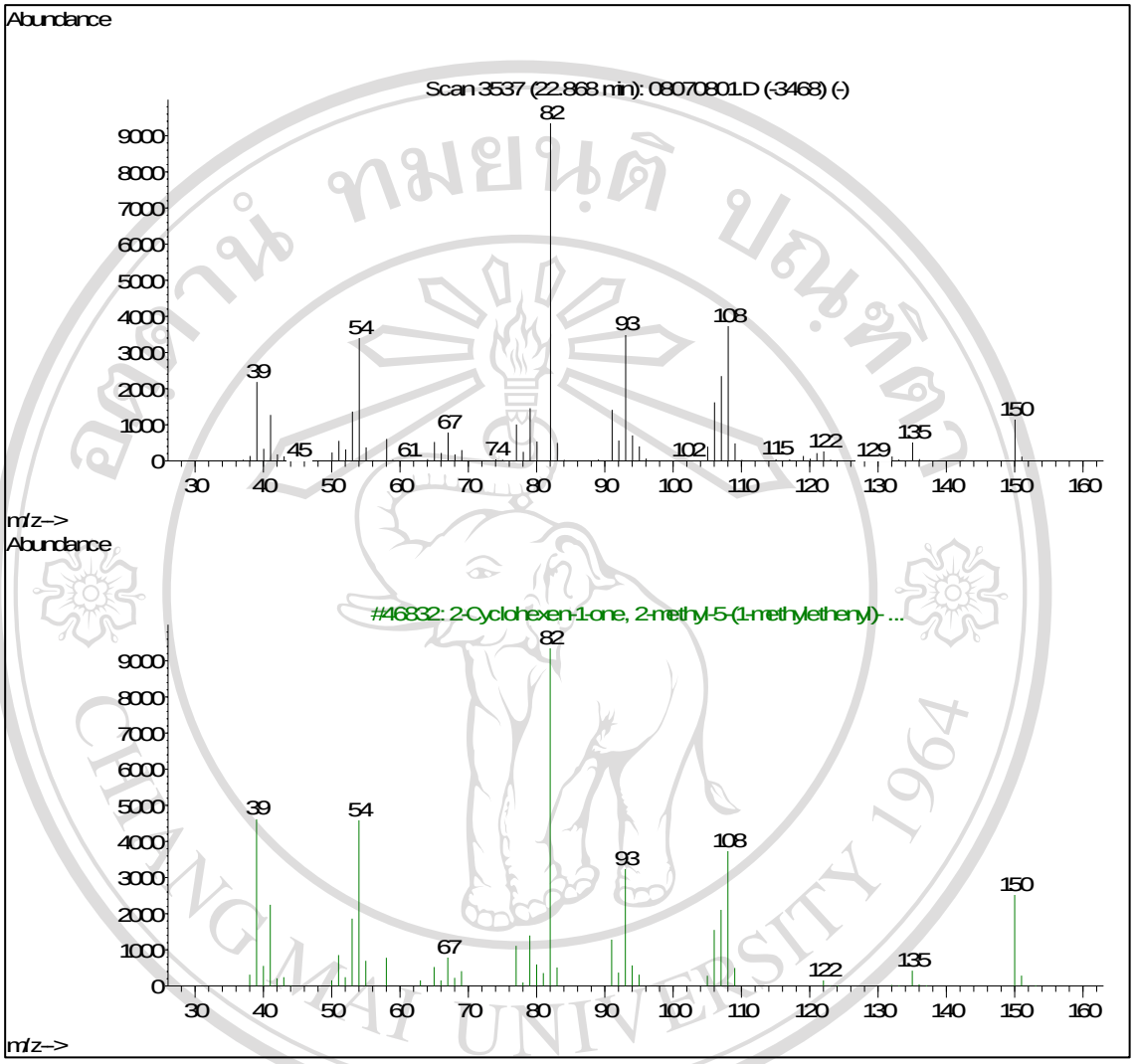
a)



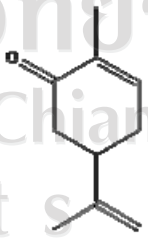
b)

B-3 a) Mass spectrum of *A. graveolens* at retention time 20.906 min (above), and reference spectrum of cyclohexanone (bottom) b) Structure of cyclohexanone

a)

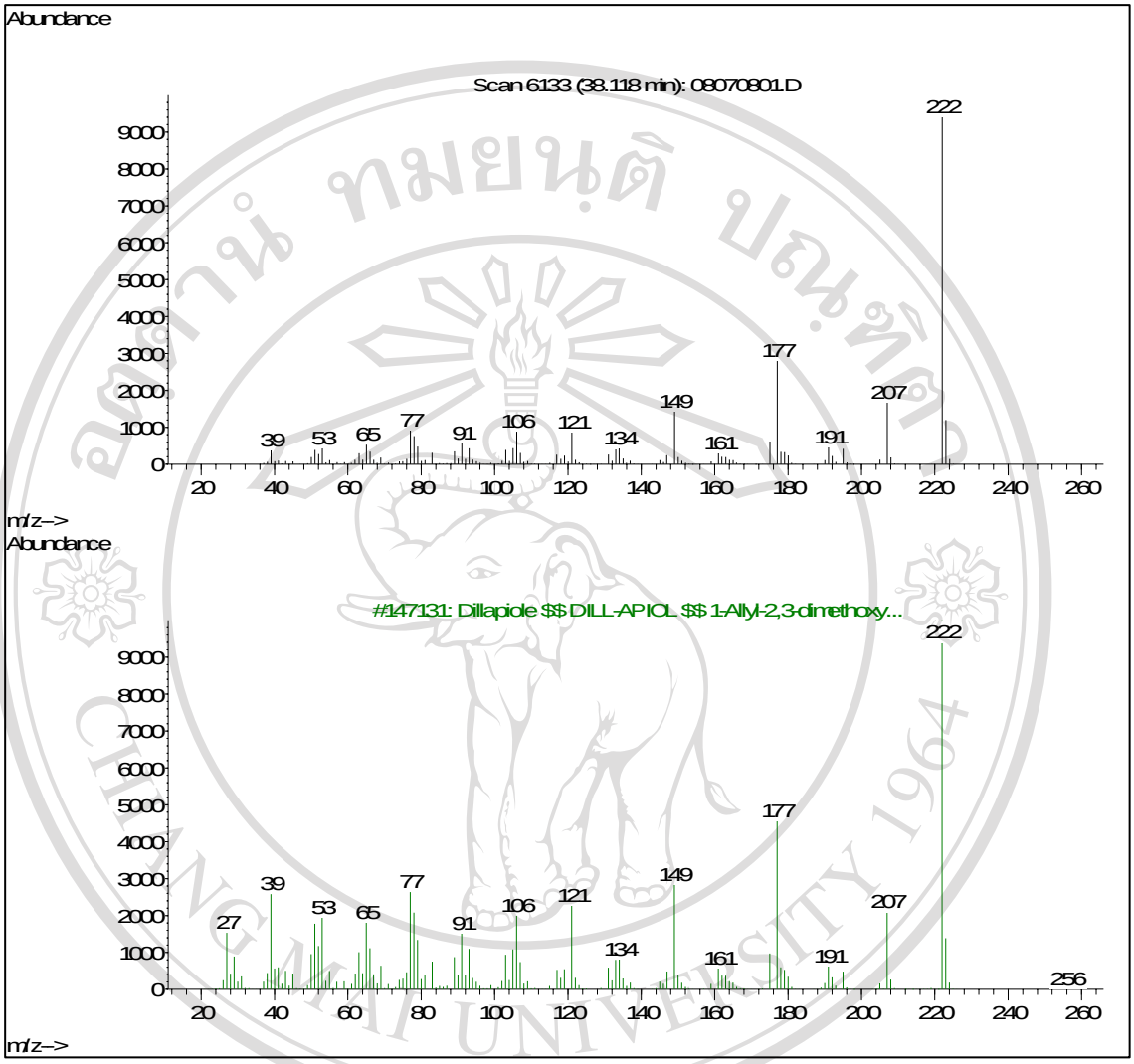


b)

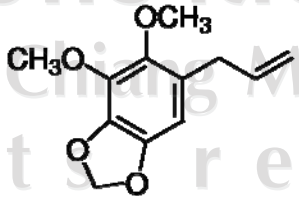


B-4 a) Mass spectrum of *A. graveolens* at retention time 22.868 min (above), and reference spectrum of 1-carvone (bottom) b) Structure of 1-carvone

a)

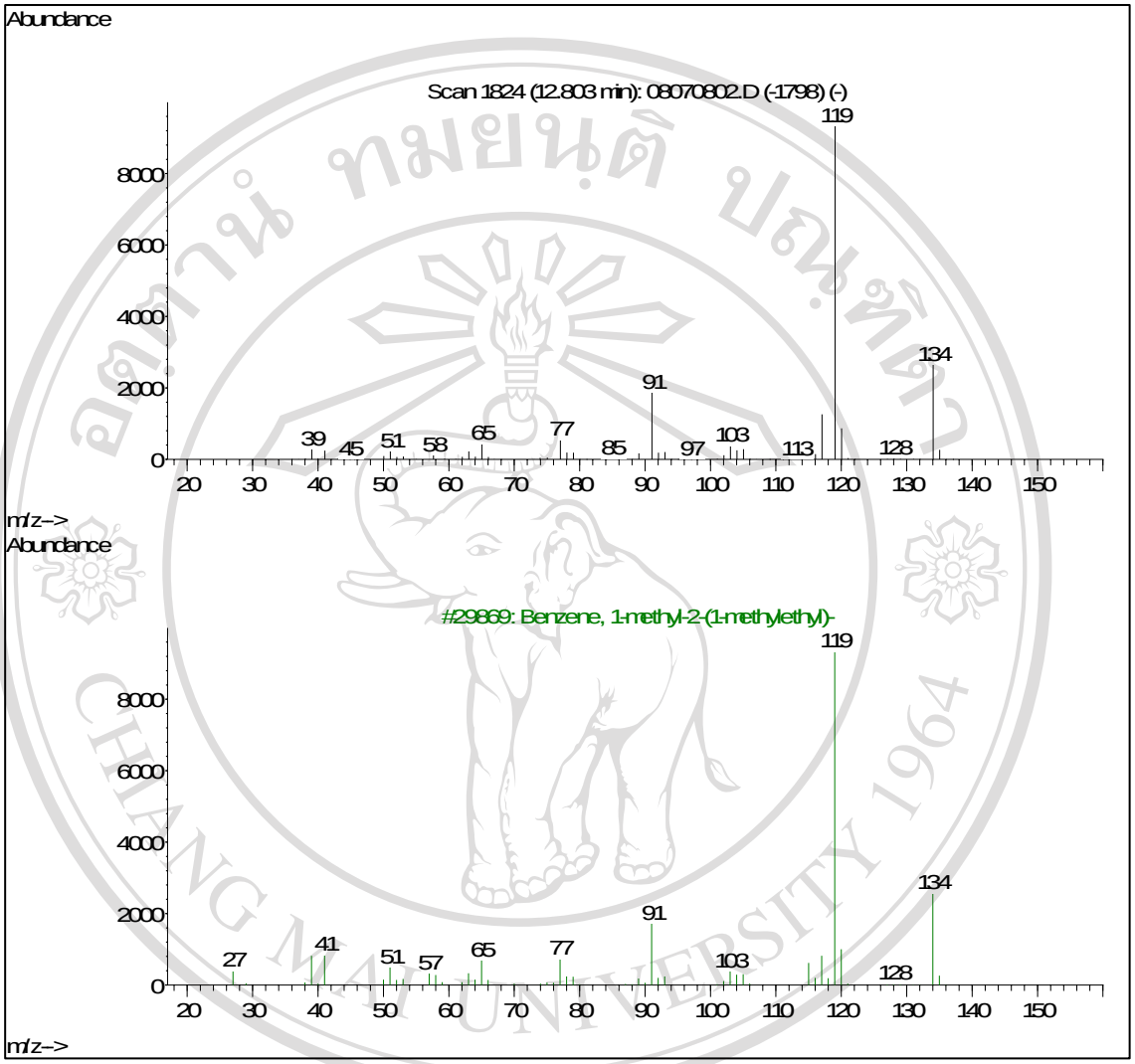


b)



B-5 a) Mass spectrum of *A. graveolens* at retention time 38.118 min (above), and reference spectrum of dillapiole (bottom) b) Structure of dillapiole

a)



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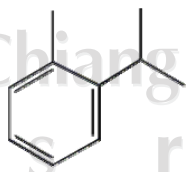
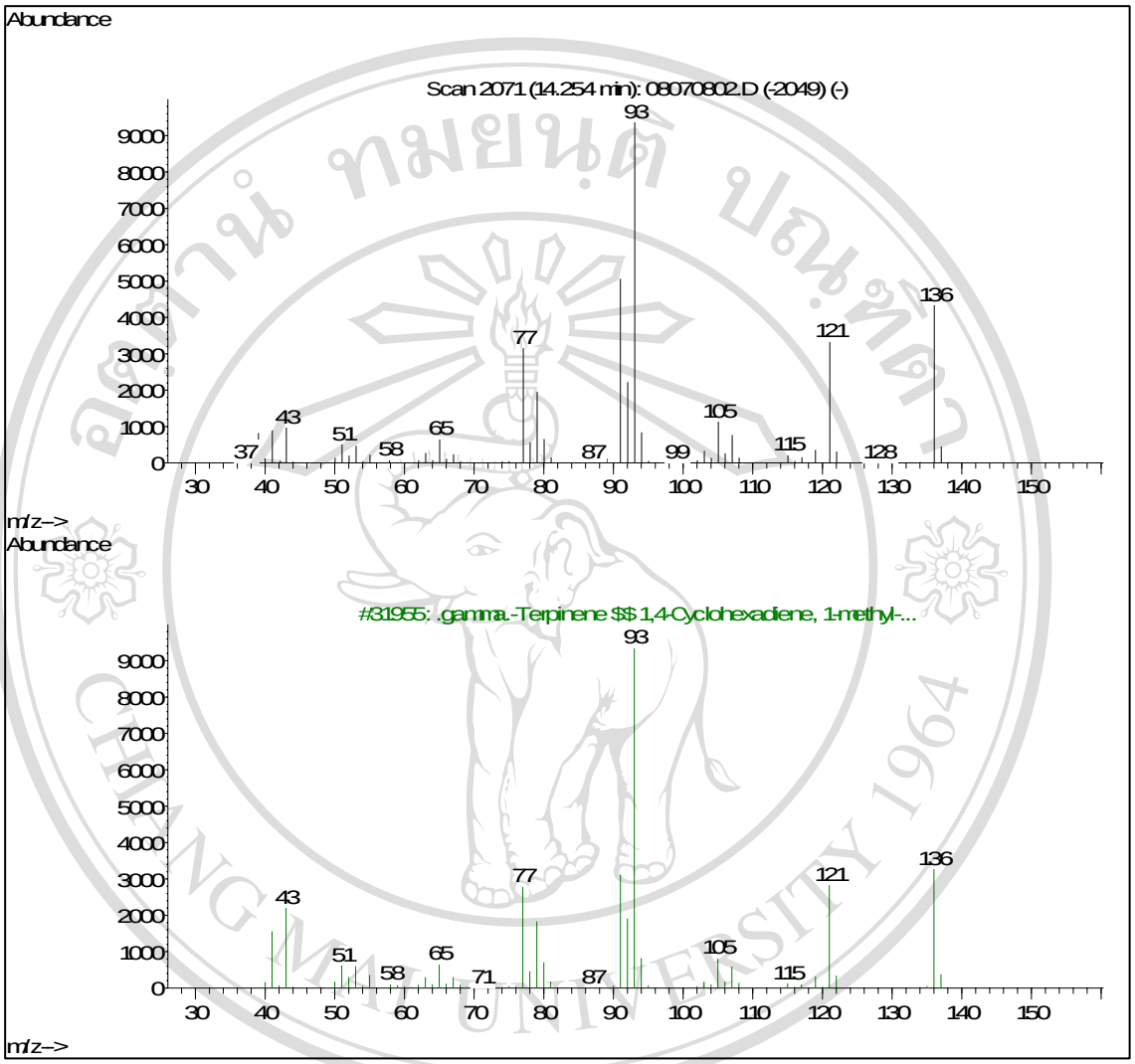


B-6 a) Mass spectrum of *C. cyminum* at retention time 12.803 min (above), and reference spectrum of 1-methyl-2-(1-methylethyl) (bottom) b) Structure of 1-methyl-2-(1-methylethyl)

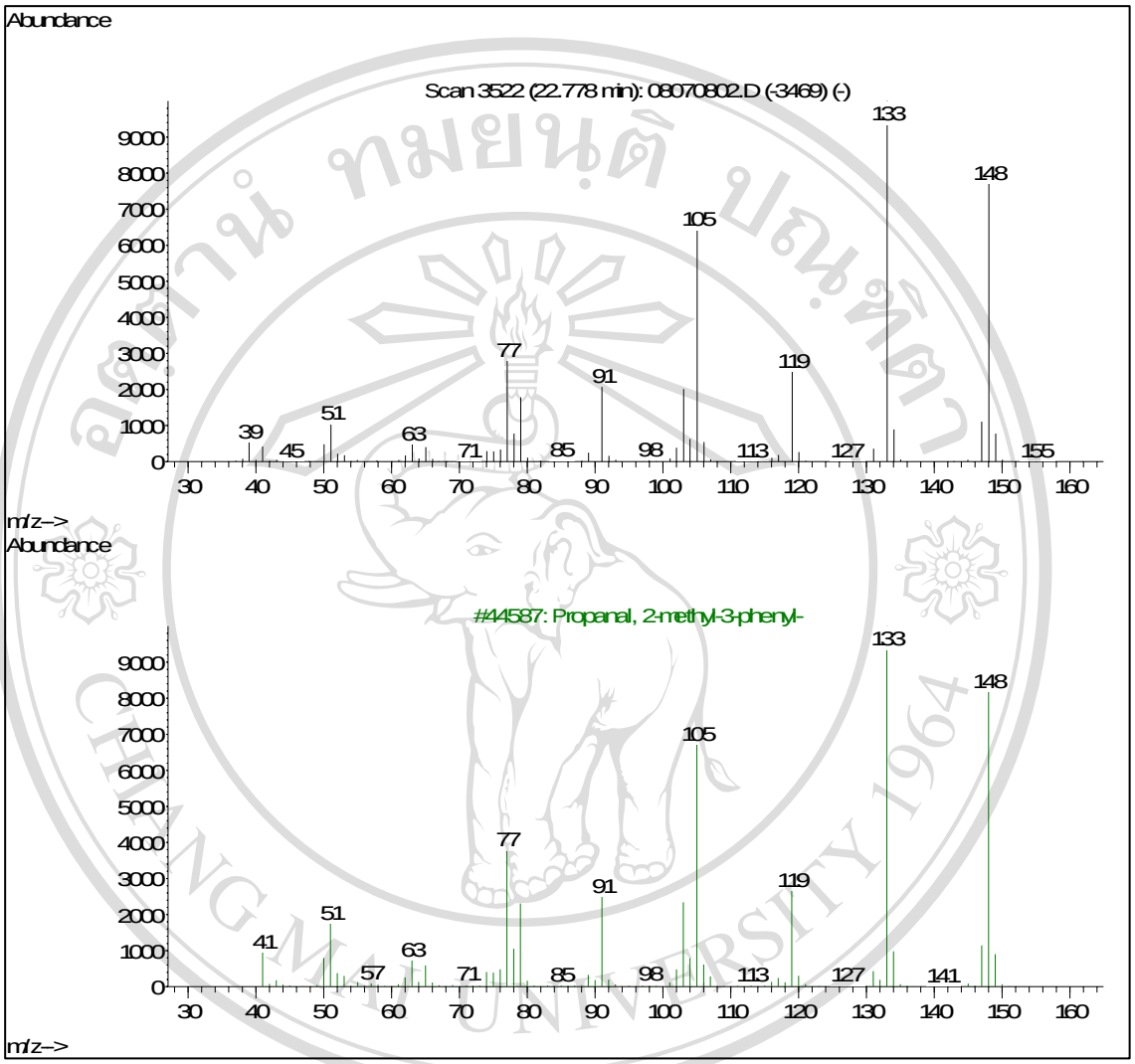
a)



b)

B-7 a) Mass spectrum of *C. cyminum* at retention time 14.254 min (above), and reference spectrum of γ-terpinene (bottom) b) Structure of γ-terpinene

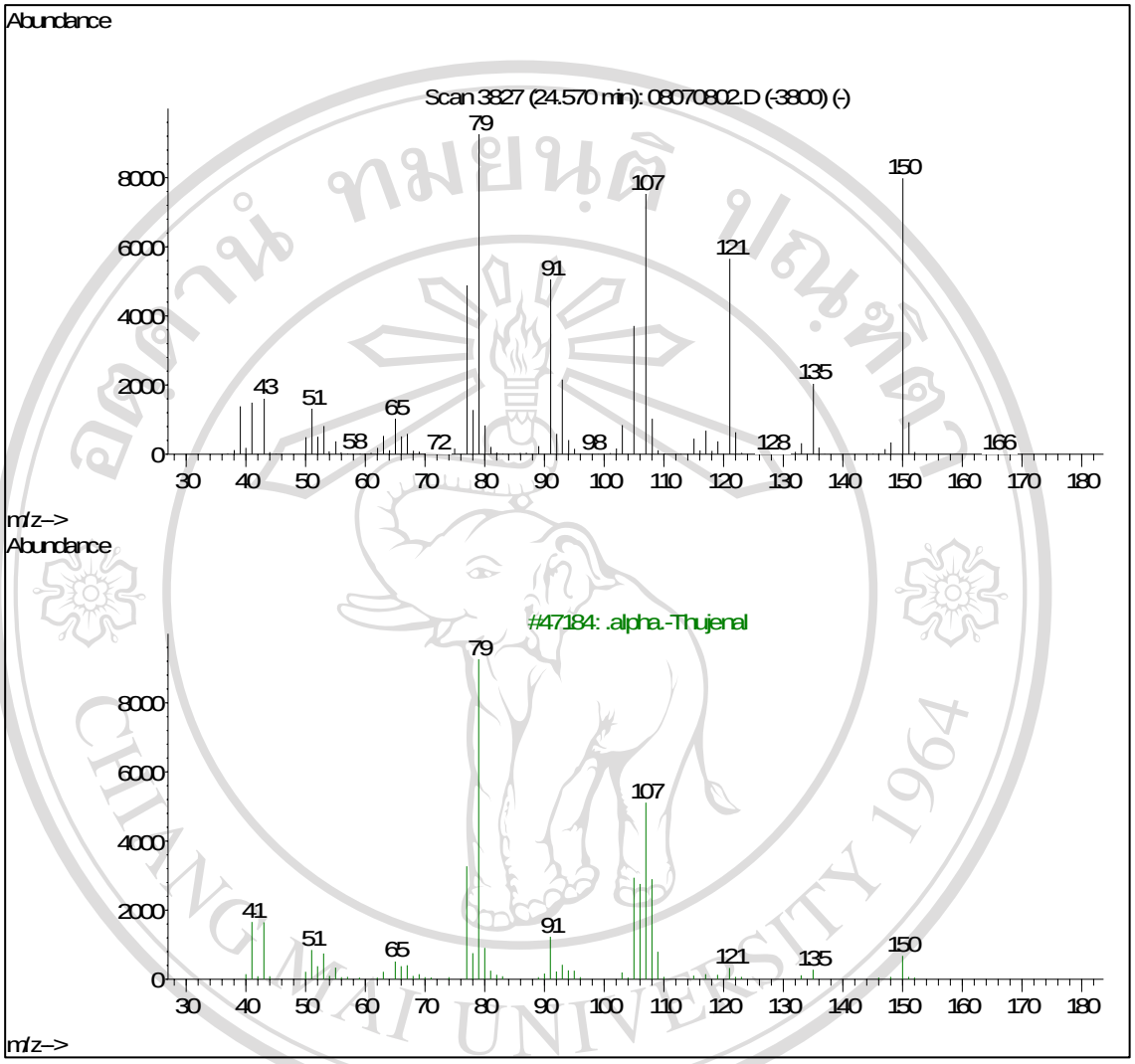
a)



b)

B-8 a) Mass spectrum of *C. cyminum* at retention time 22.778 min (above), and reference spectrum of cuminic aldehyde (bottom) b) Structure of cuminic aldehyde

a)



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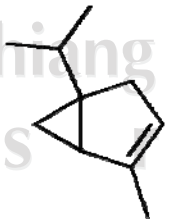
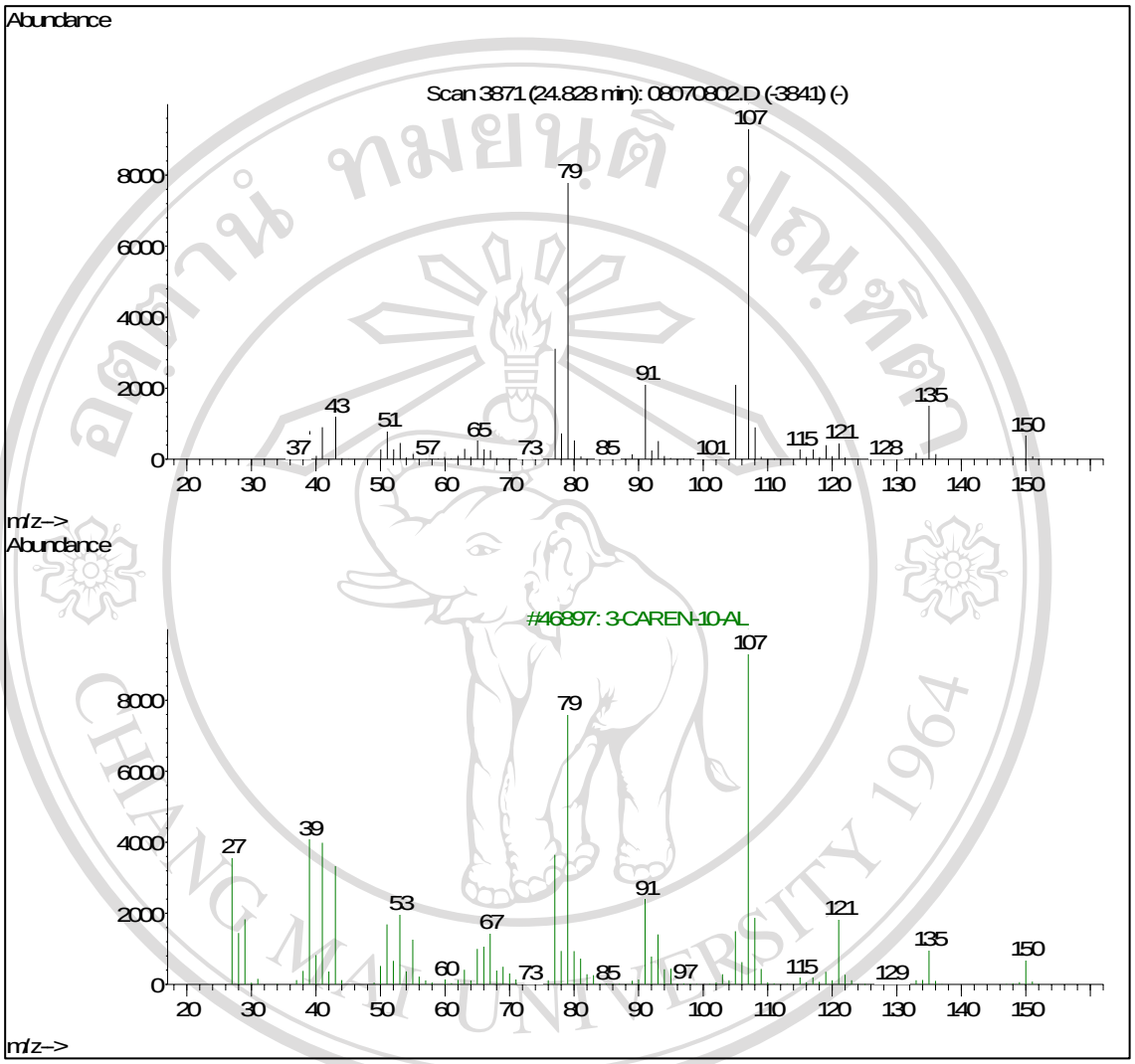


B-9 a) Mass spectrum of *C. cyminum* at retention time 24.570 min (above), and reference spectrum of α-thujenal (bottom) b) Structure of α-thujenal

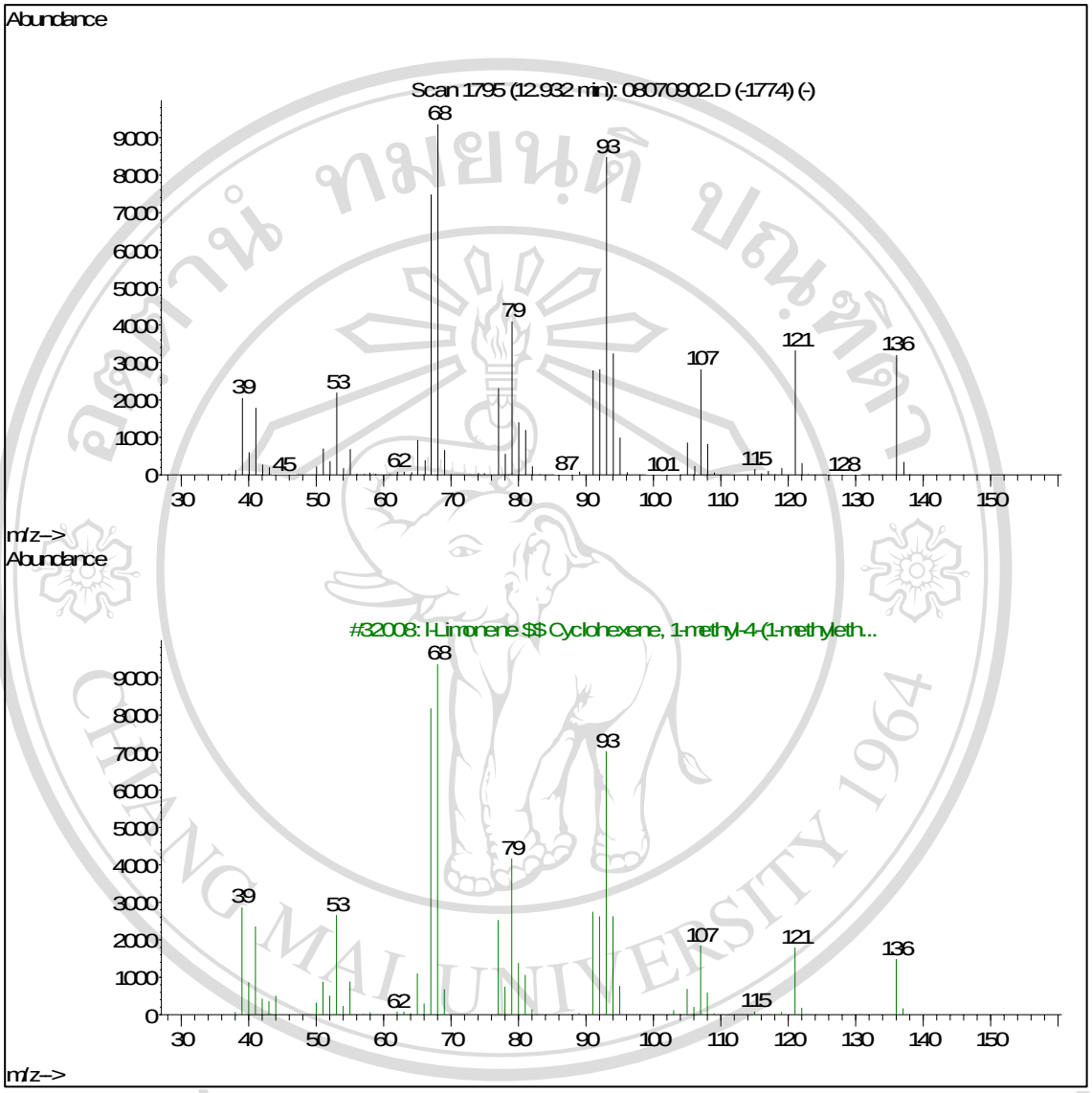
a)



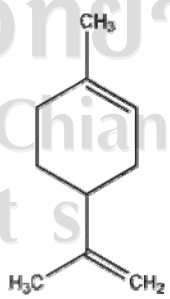
b)

B-10 a) Mass spectrum of *C. cyminum* at retention time 24.828 min (above), and reference spectrum of 3-caren-10-al (bottom) b) Structure of 3-caren-10-al

a)

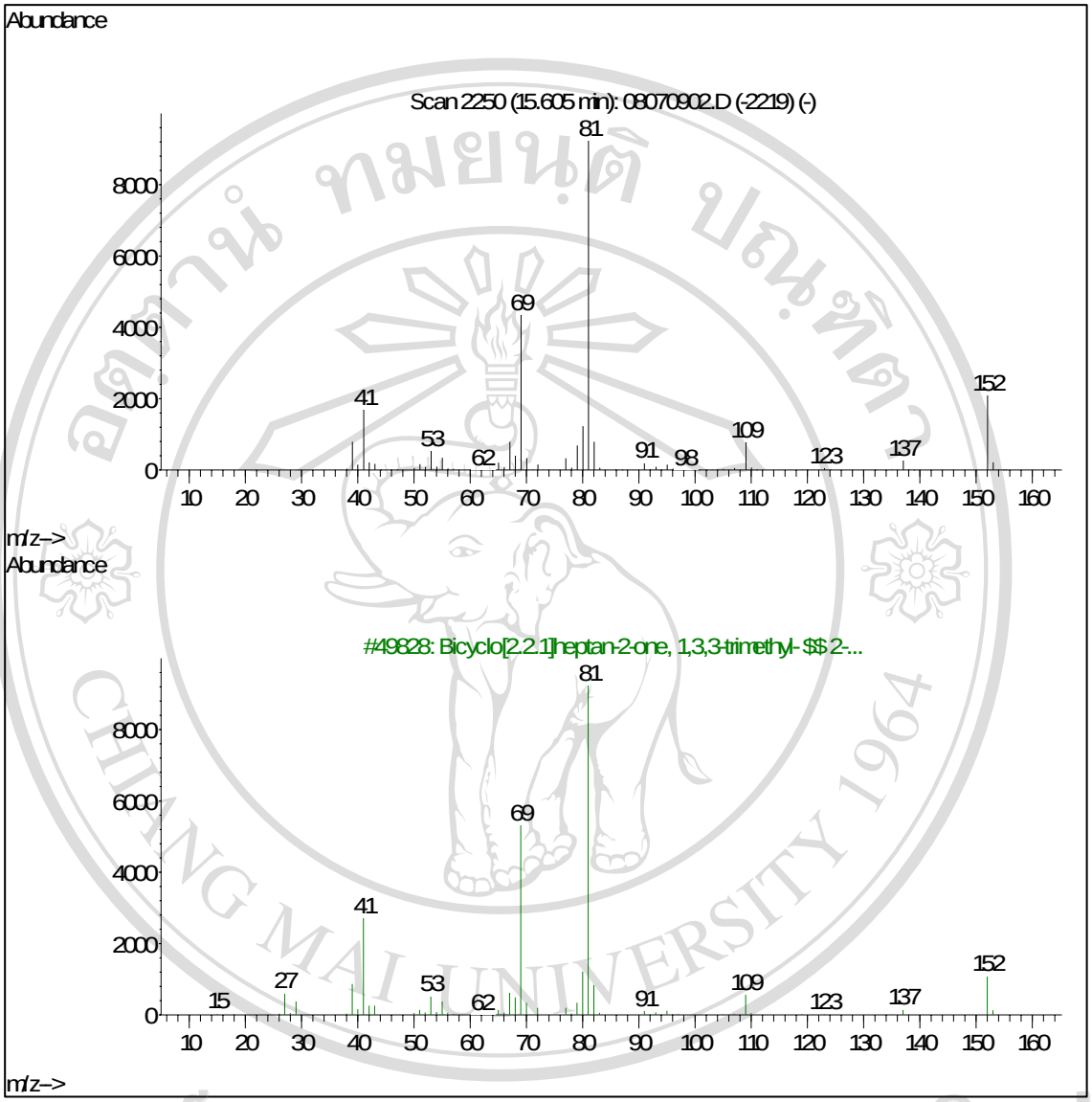


b)



B-11 a) Mass spectrum of *F. vulgare* at retention time 12.932 min (above), and reference spectrum of dl-limonene (bottom) b) Structure of dl-limonene

a)



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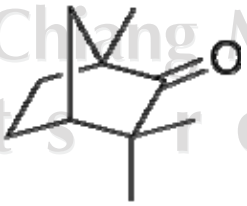
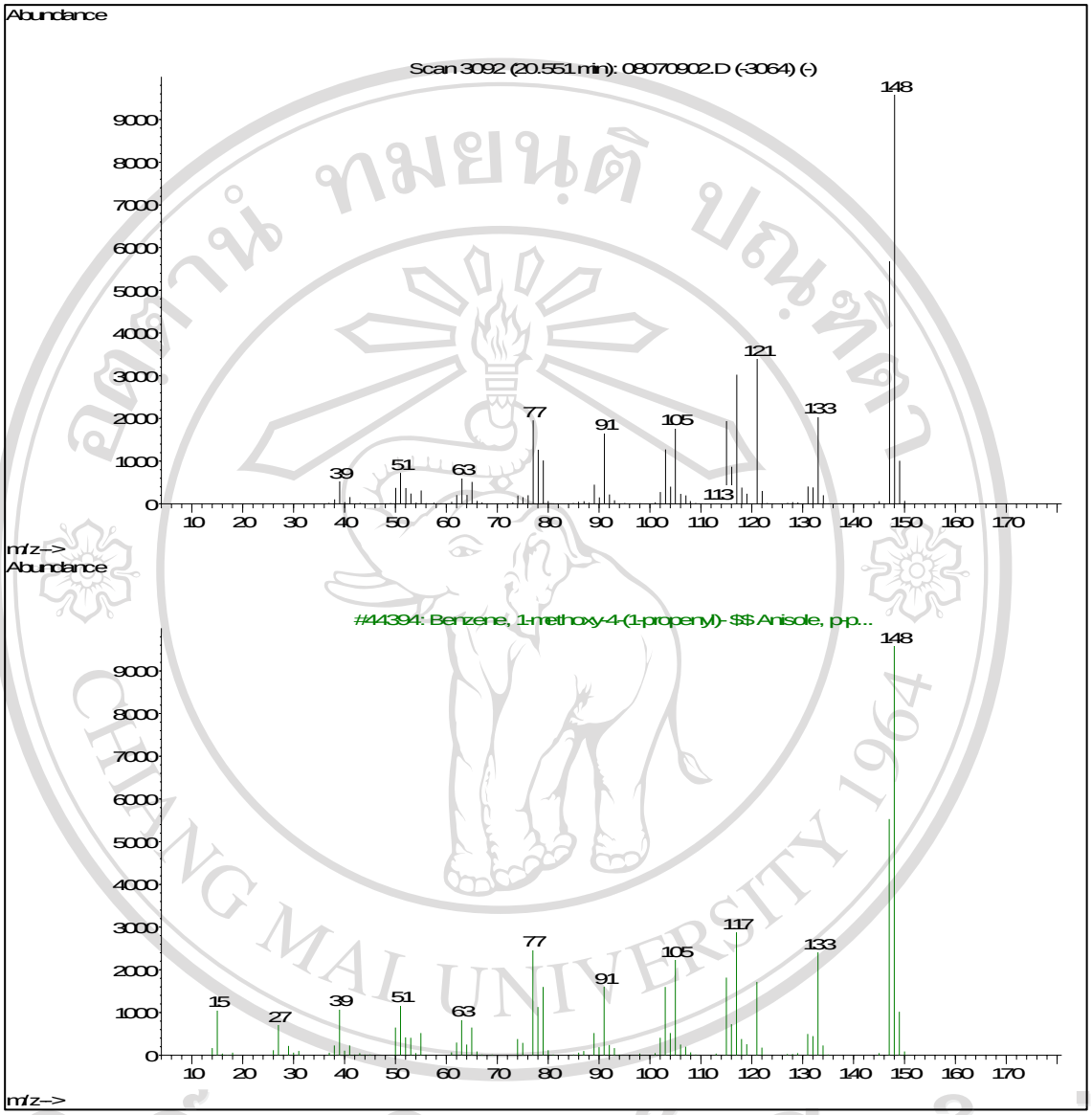


B-12 a) Mass spectrum of *F. vulgare* at retention time 15.605 min (above), and reference spectrum of α-fenchone (bottom) b) Structure of α-fenchone

a)



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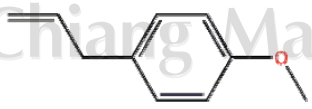
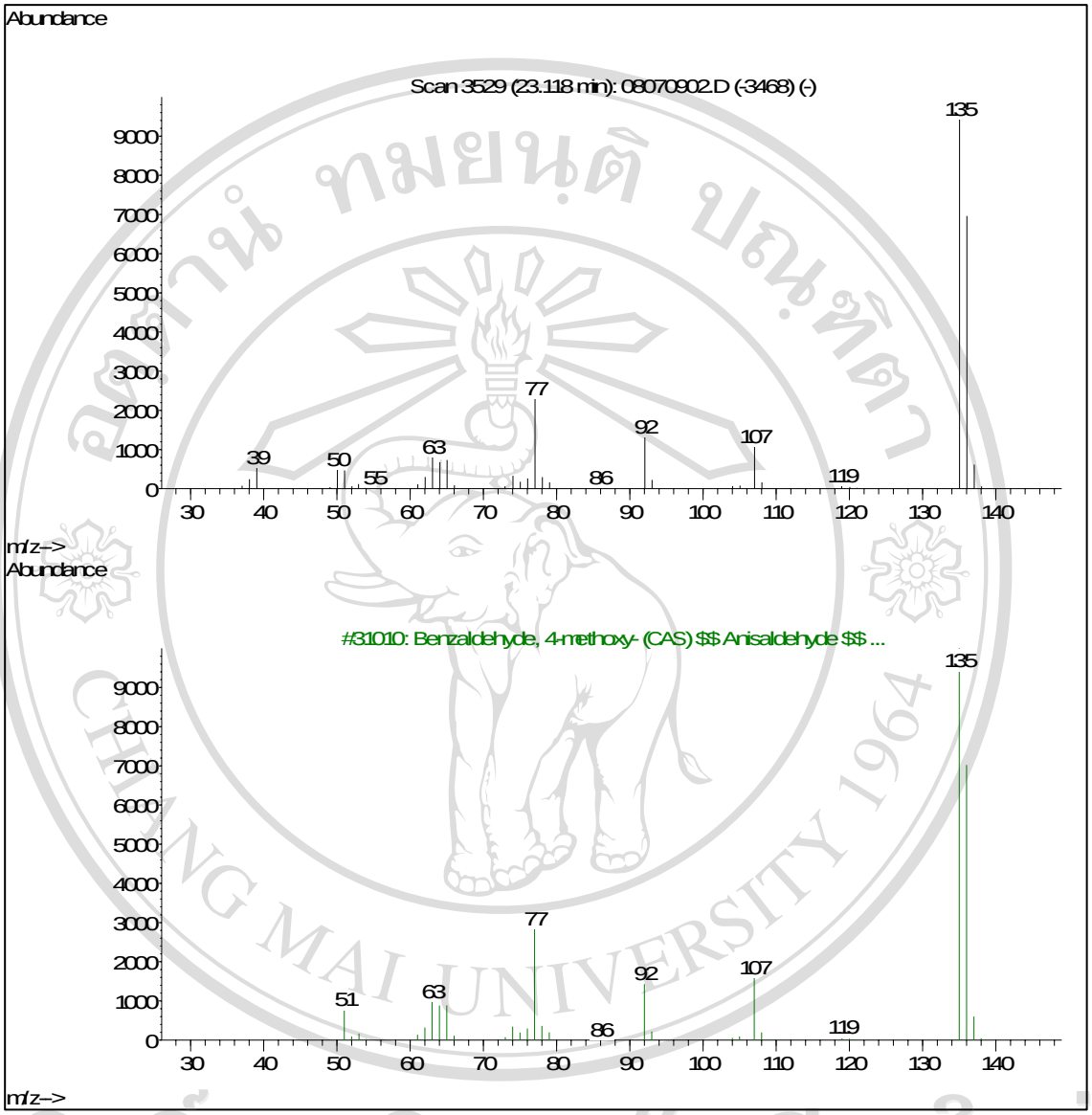
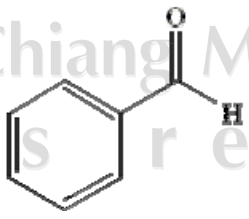


B-13 a) Mass spectrum of *F. vulgare* at retention time 20.551 min (above), and reference spectrum of 1-methoxy-4-(2-propenyl) (bottom) b) Structure of 1-methoxy-4-(2-propenyl)

a)

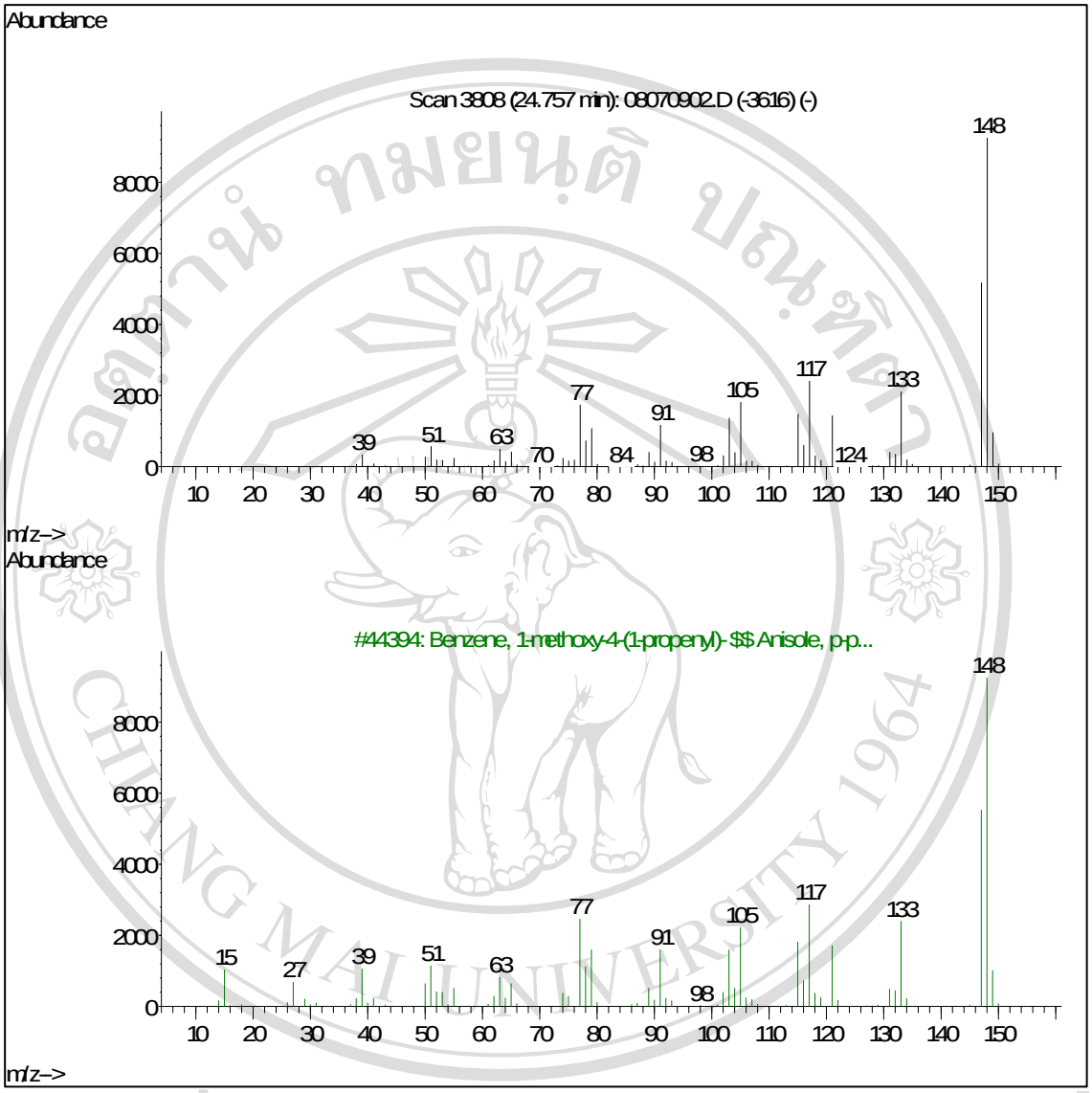


b)

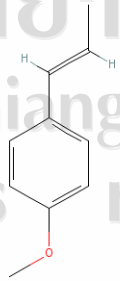


B-14 a) Mass spectrum of *F. vulgare* at retention time 23.118 min (above), and reference spectrum of benzaldehyde (bottom) b) Structure of benzaldehyde

a)

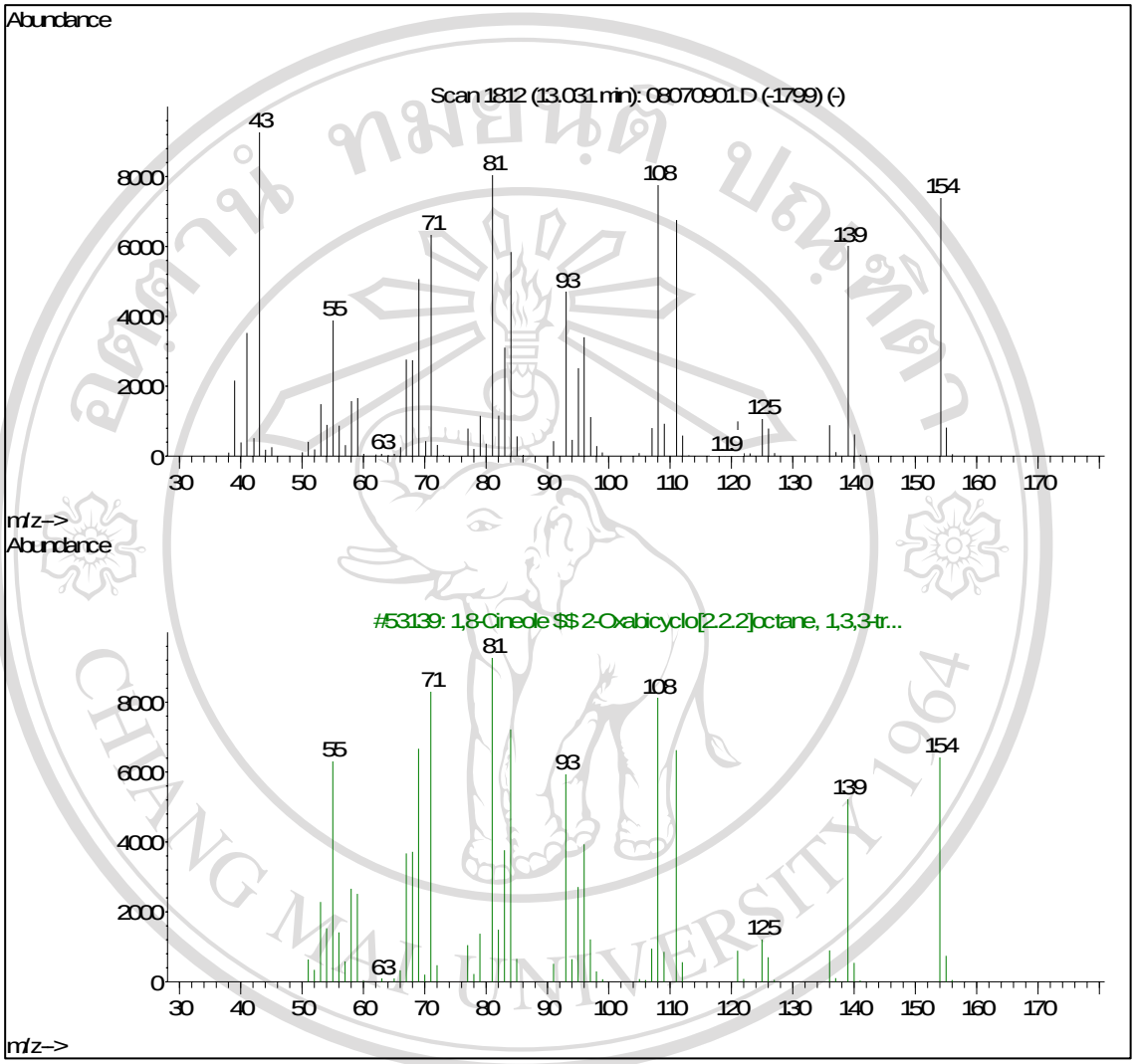


b)

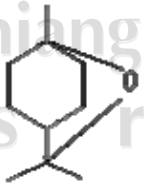


B-15 a) Mass spectrum of *F. vulgare* at retention time 24.757 min (above), and reference spectrum of *trans*-anethole (bottom) b) Structure of *trans*-anethole

a)

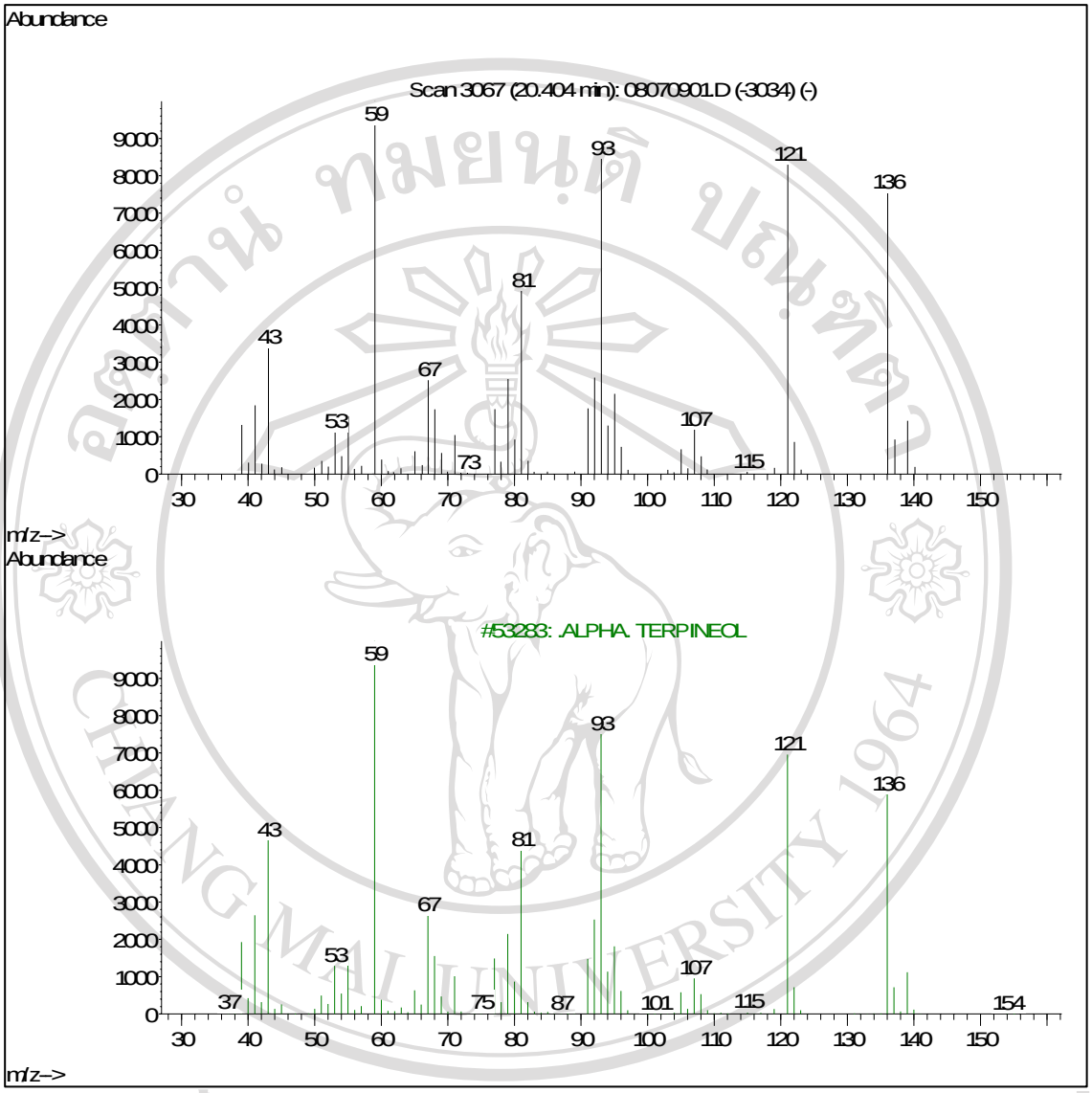


b)

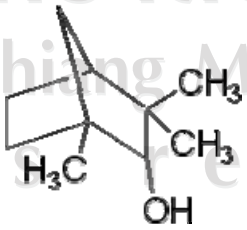


B-16 a) Mass spectrum of *Cinnamomum* sp. at retention time 13.013 min (above), and reference spectrum of 1, 8-cineole (bottom) b) Structure of 1, 8-cineole

a)

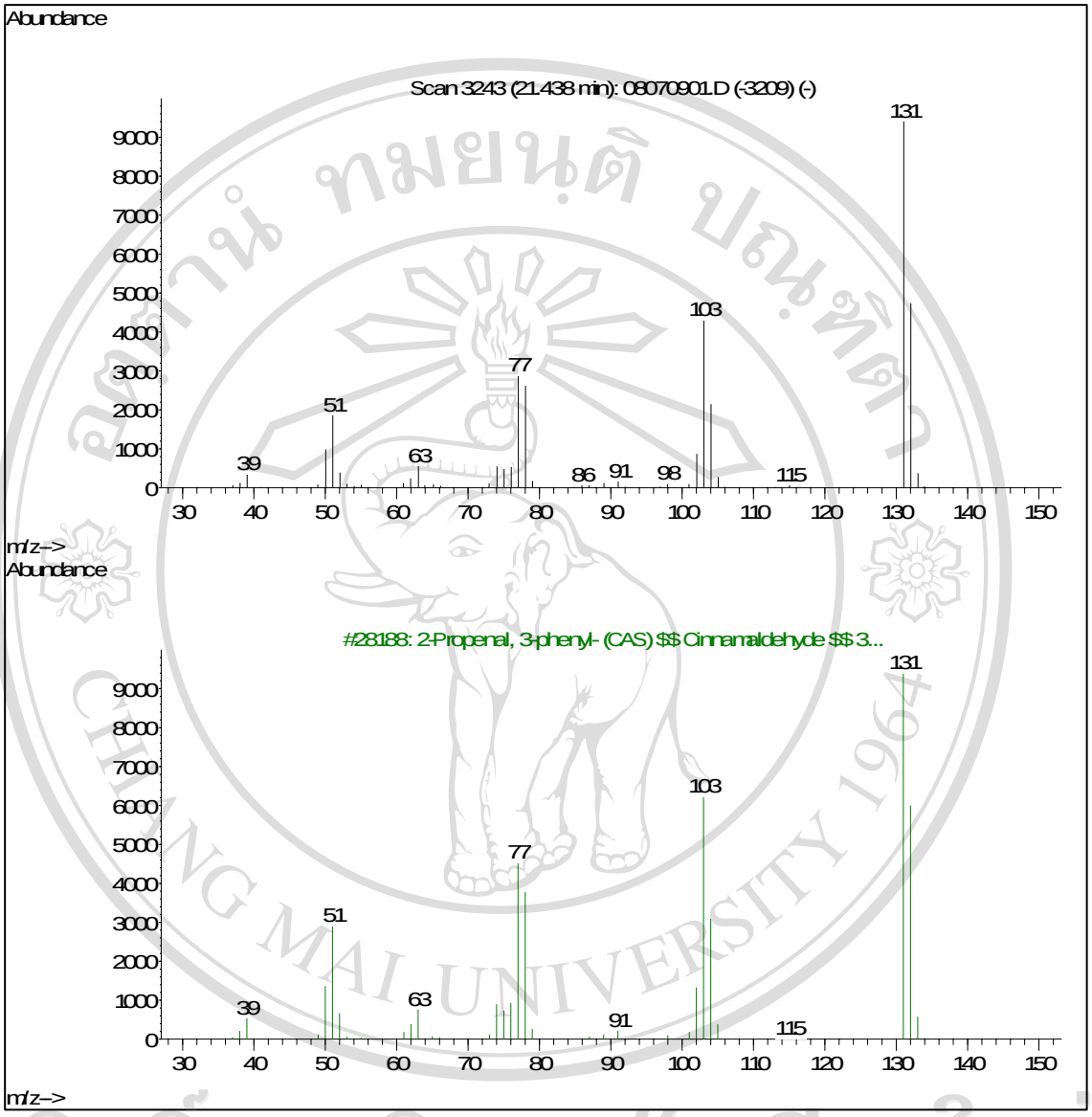


b)



B-17 a) Mass spectrum of *Cinnamomum* sp. at retention time 20.404 min (above), and reference spectrum of β-fenchyl alcohol (bottom) b) Structure of β-fenchyl alcohol

a)



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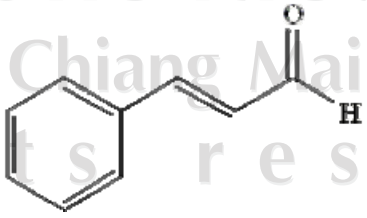
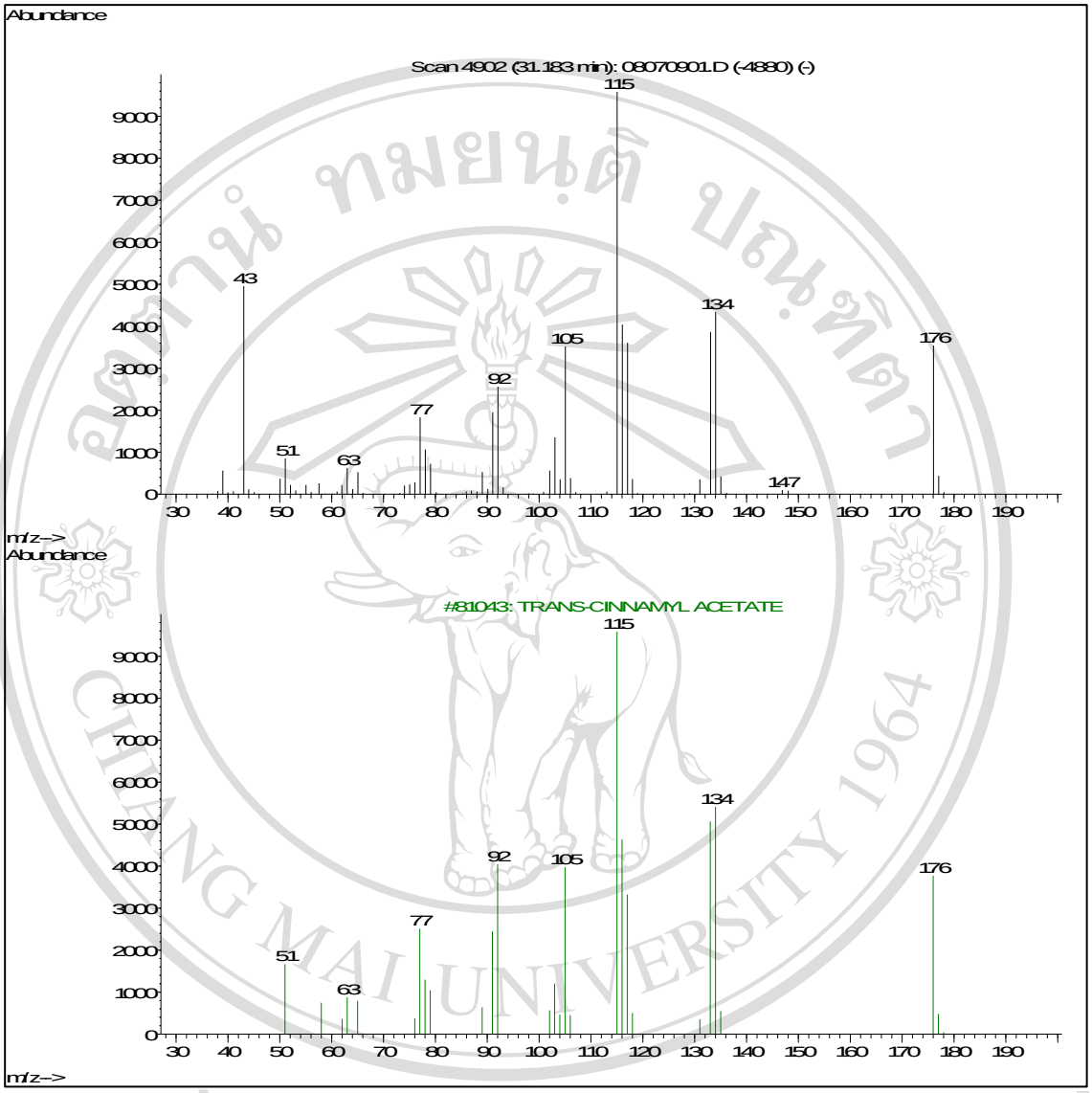


B-18 a) Mass spectrum of *Cinnamomum* sp. at retention time 21.438 min (above), and reference spectrum of cinnamaldehyde (bottom) b) Structure of cinnamaldehyde

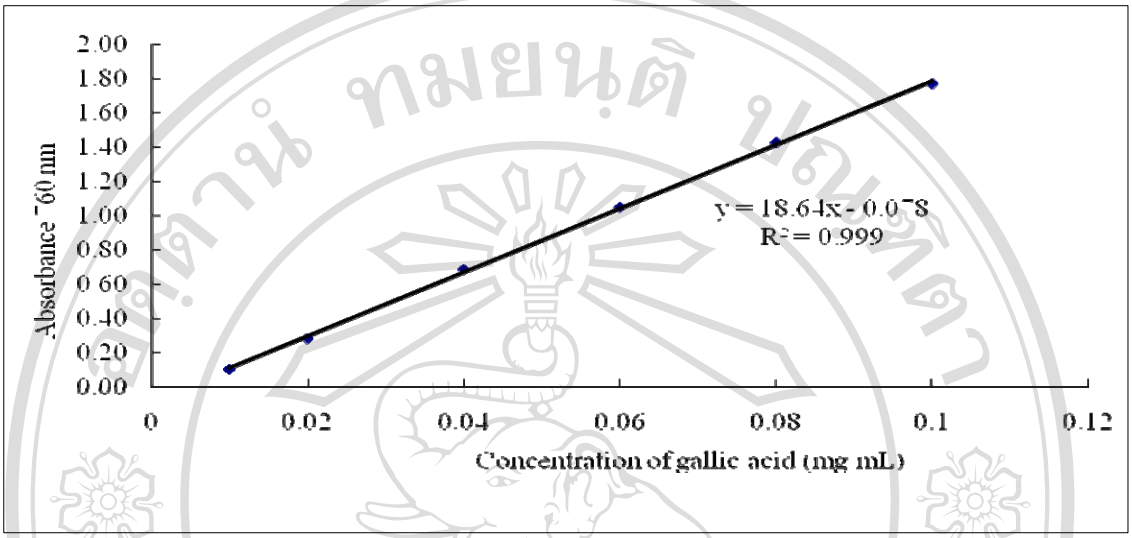
a)



b)

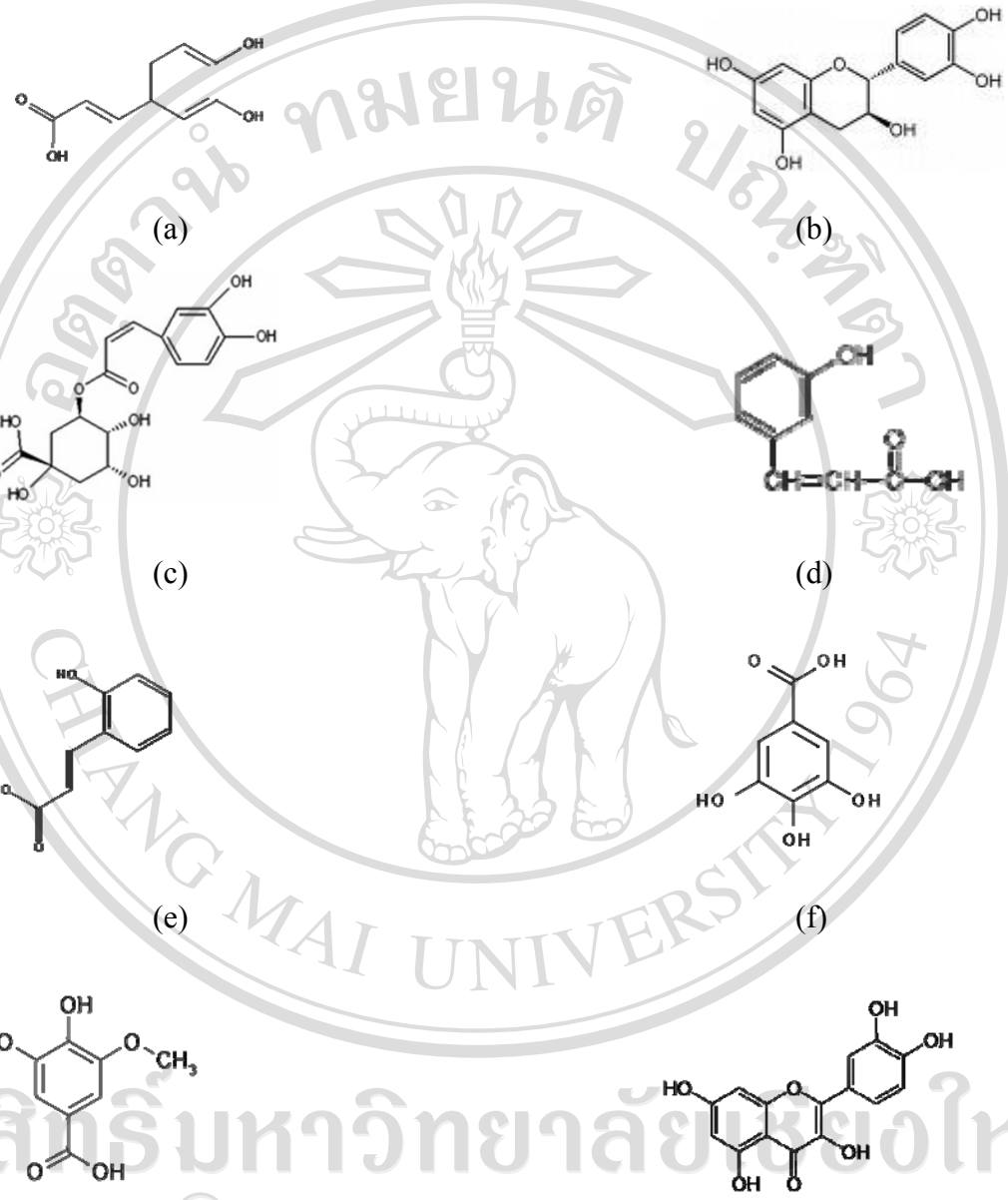
B-19 a) Mass spectrum of *Cinnamomum* sp. at retention time 31.183 min (above), and reference spectrum of *trans*-cinnamyl acetate (bottom) b) Structure of *trans*-cinnamyl acetate

C. Calibration curve of total phenolic content analysis



C-1 Calibration curve of gallic acid solution

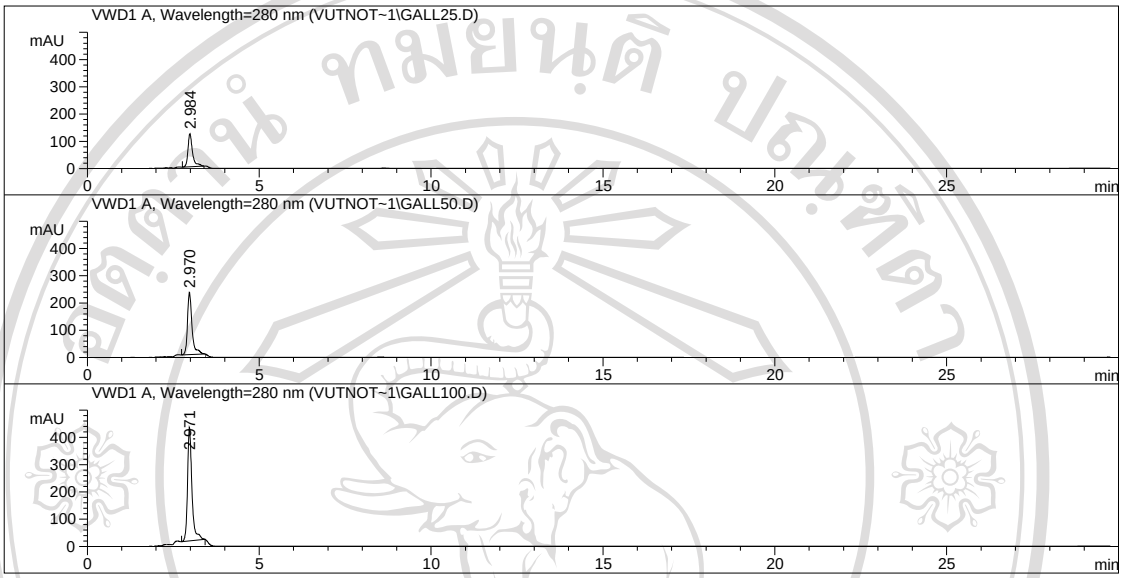
D. Structure of standard polyphenolic compounds



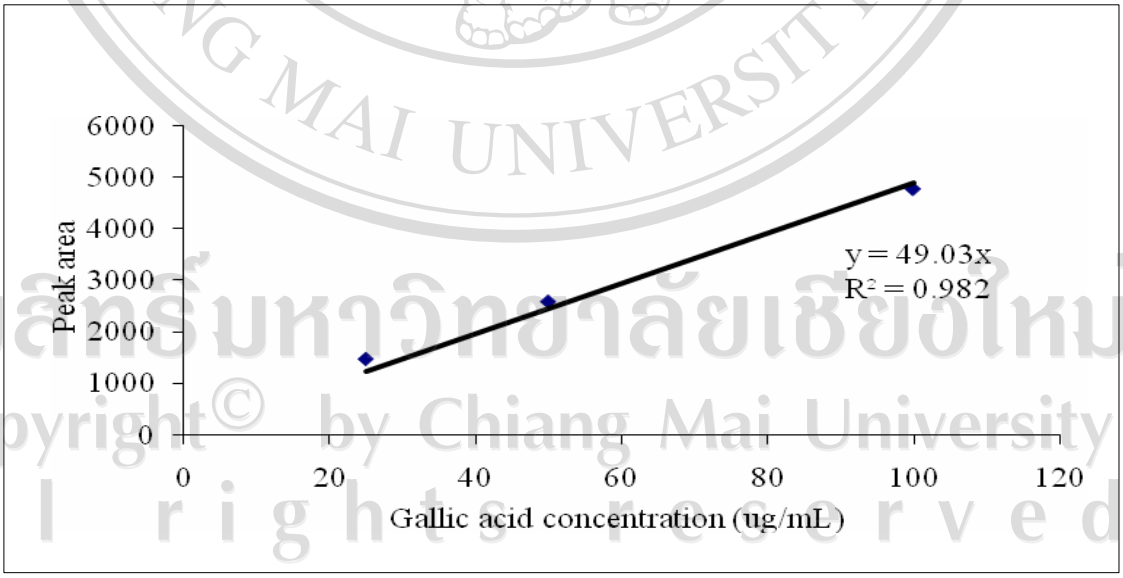
D-1 Structure of standard polyphenolic compounds a) caffeic acid b) catechin c) chlorogenic acid d) m-coumaric acid e) o-coumaric acid f) gallic acid g) syringic acid and h) quercetin.

E. HPLC chromatogram of polyphenolic standards and calibration curve

a)

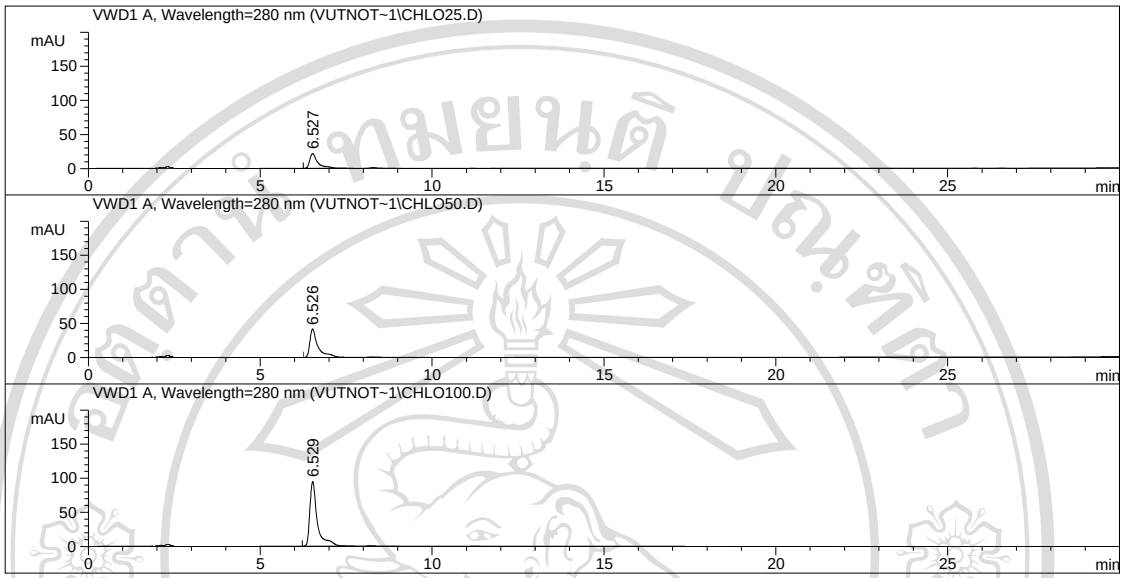


b)

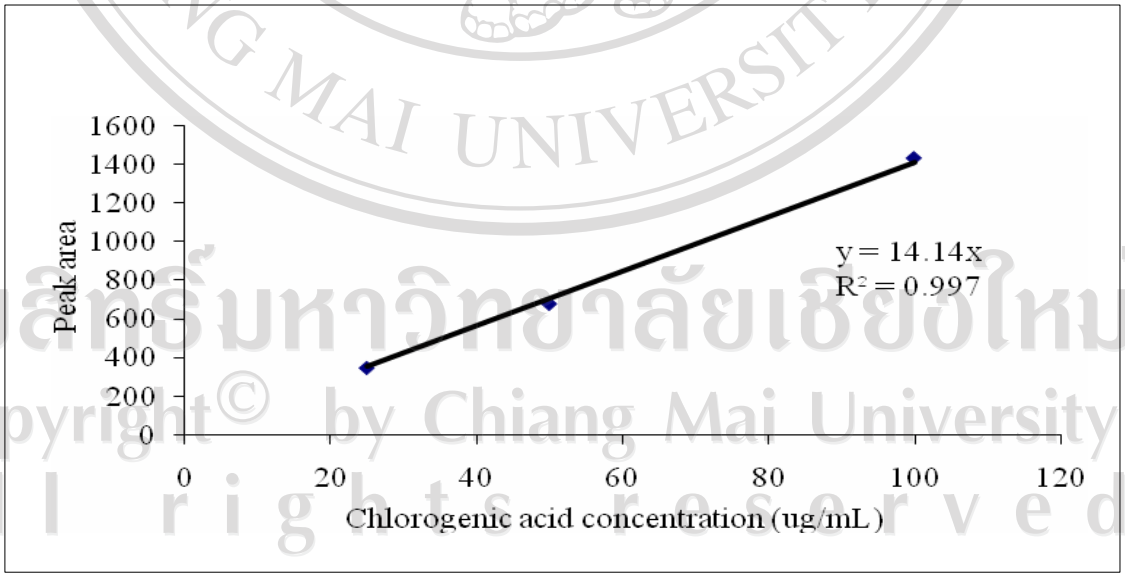


E-1 HPLC chromatograms of (a) gallic acid 25 µg/mL, gallic acid 50 µg/mL and gallic acid 100 µg/mL (b) calibration curve of gallic acid

a)

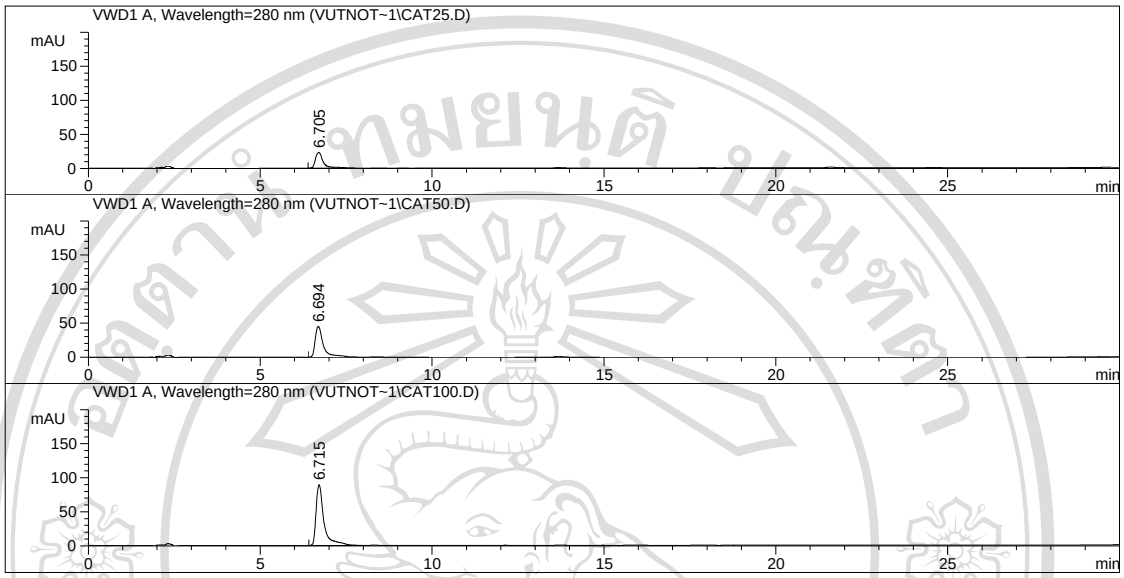


b)

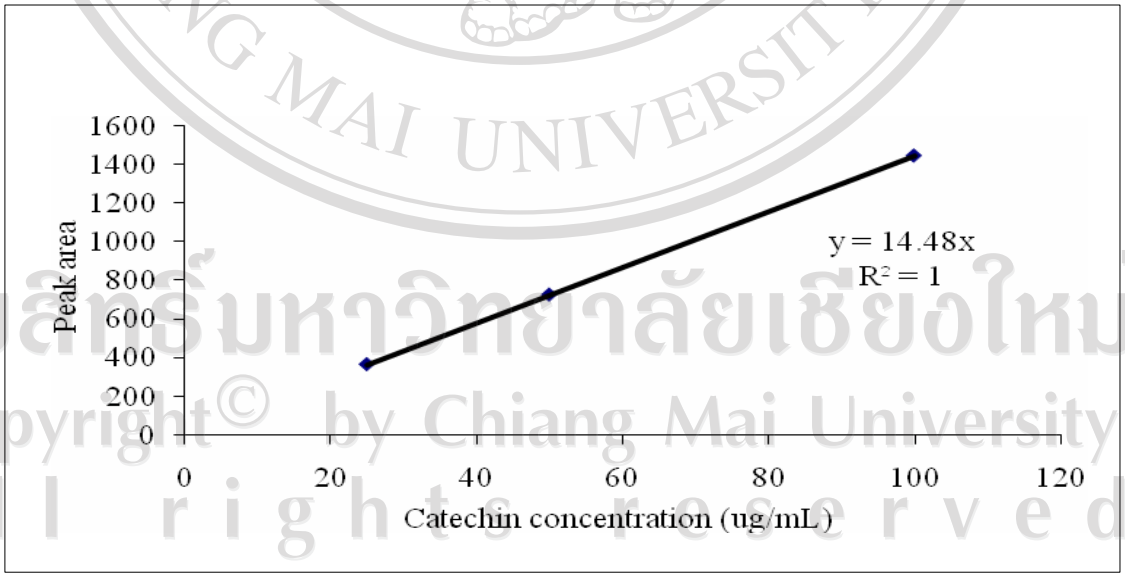


E-2 HPLC chromatograms of (a) chlorogenic acid 25 µg/mL, chlorogenic acid 50 µg/mL and chlorogenic acid 100 µg/mL (b) calibration curve of chlorogenic acid

a)

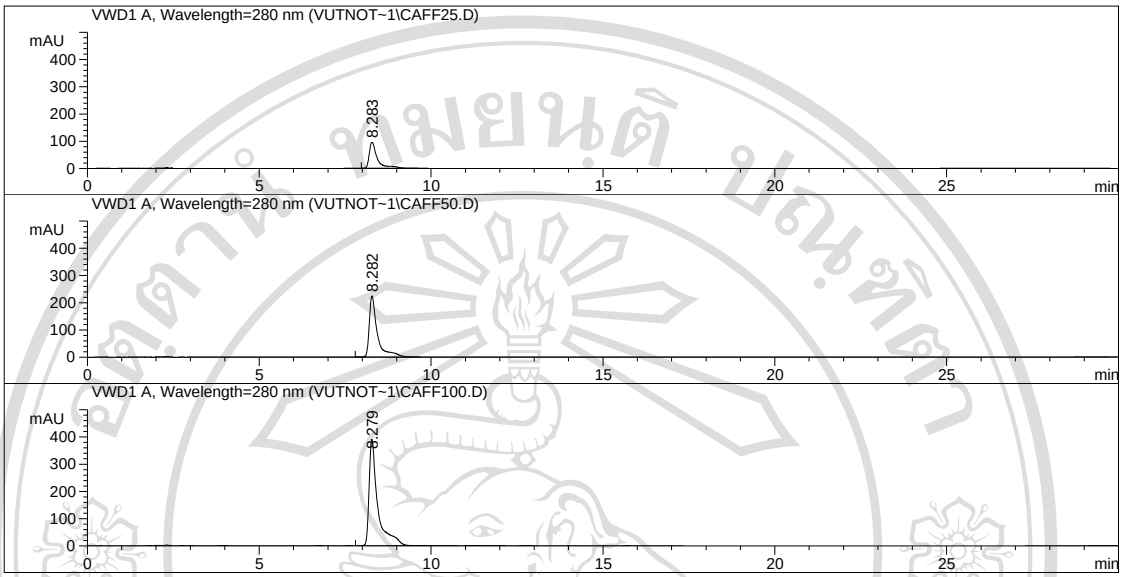


b)



E-3 HPLC chromatograms of (a) catechin 25 µg/mL, catechin 50 µg/mL and catechin 100 µg/mL (b) calibration curve of catechin

a)



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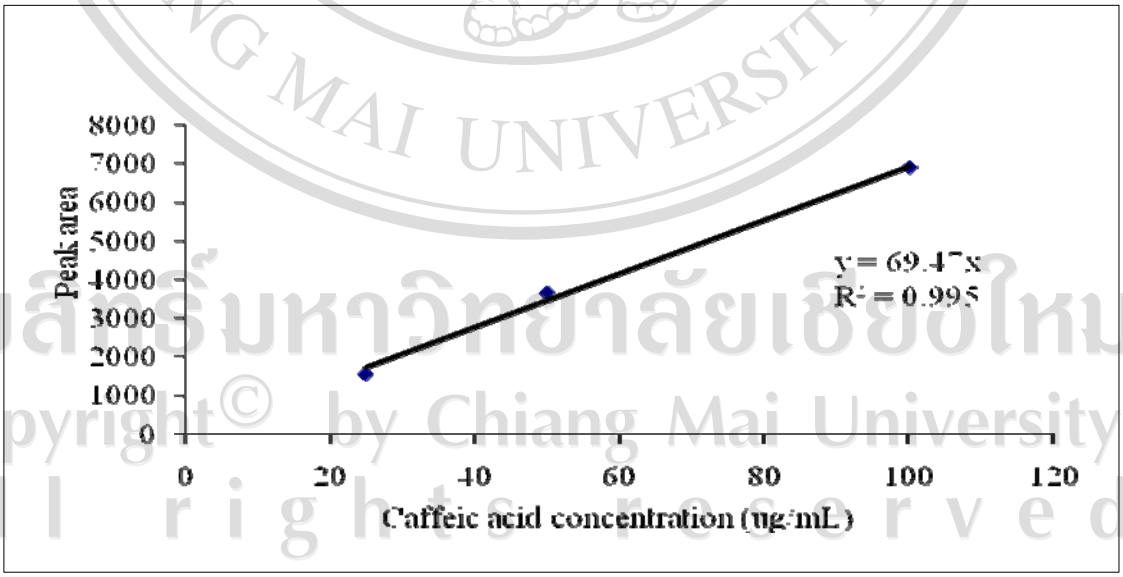
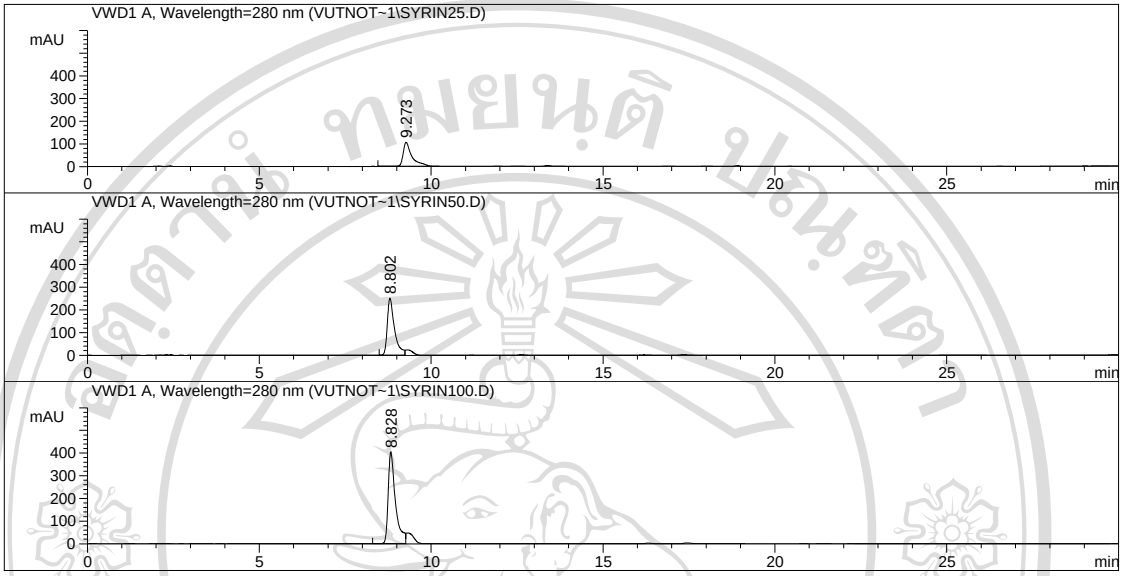
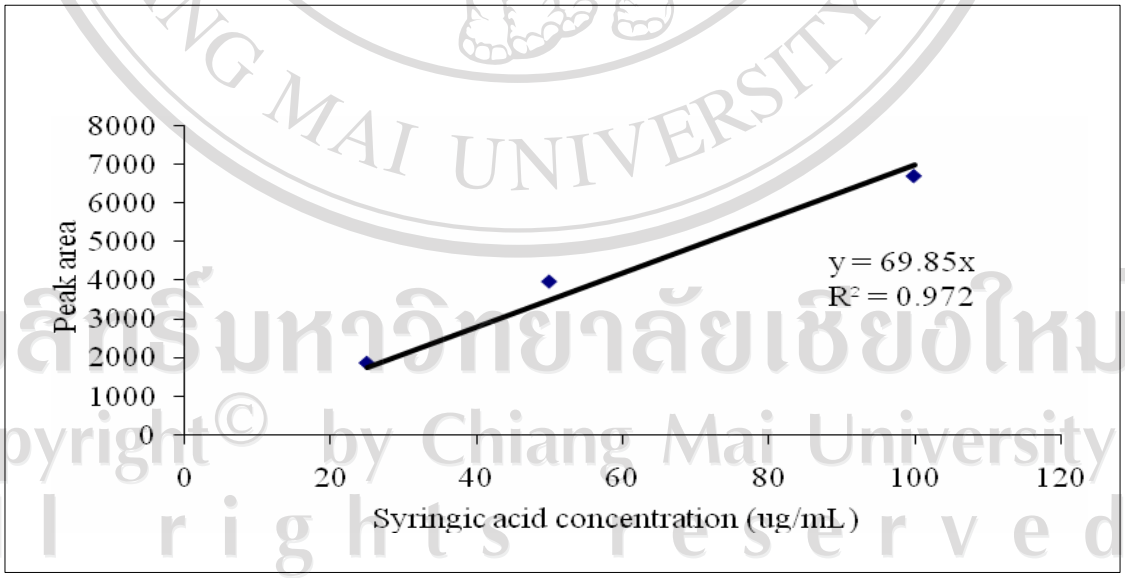


E-4 HPLC chromatograms of (a) caffeic acid 25 µg/mL, caffeic acid 50 µg/mL and caffeic acid 100 µg/mL (b) calibration curve of caffeic acid

a)

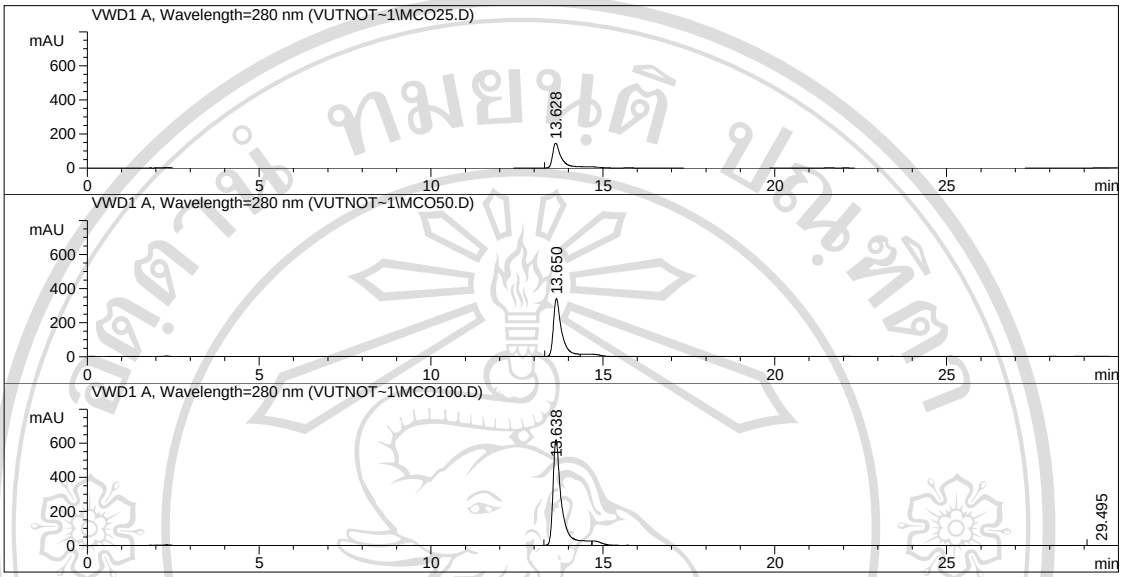


b)

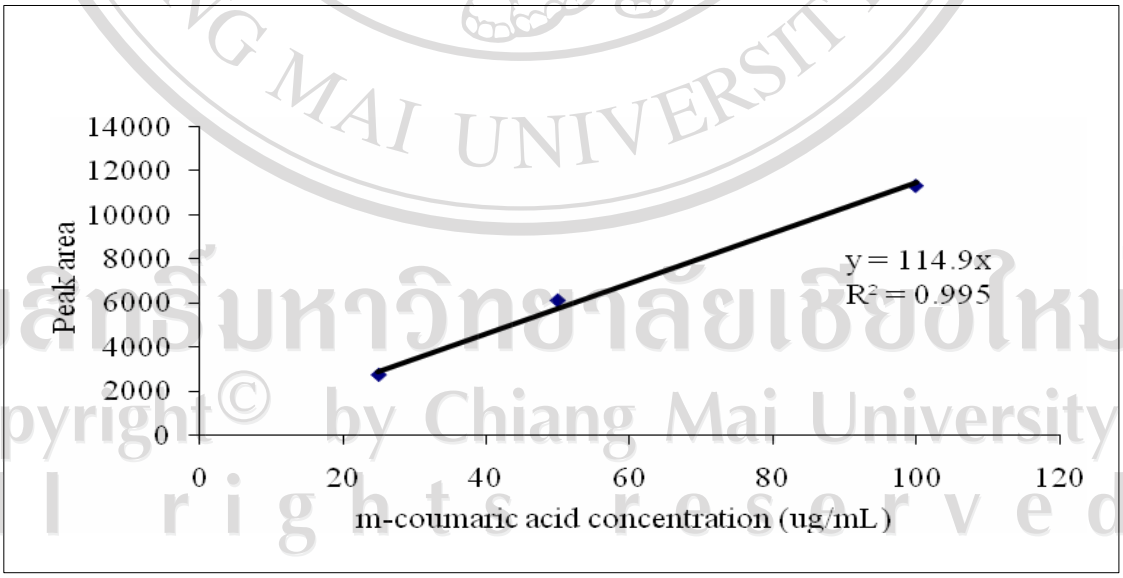


E-5 HPLC chromatograms of (a) syringic acid 25 µg/mL, syringic acid 50 µg/mL and syringic acid 100 µg/mL (b) calibration curve of syringic acid

a)

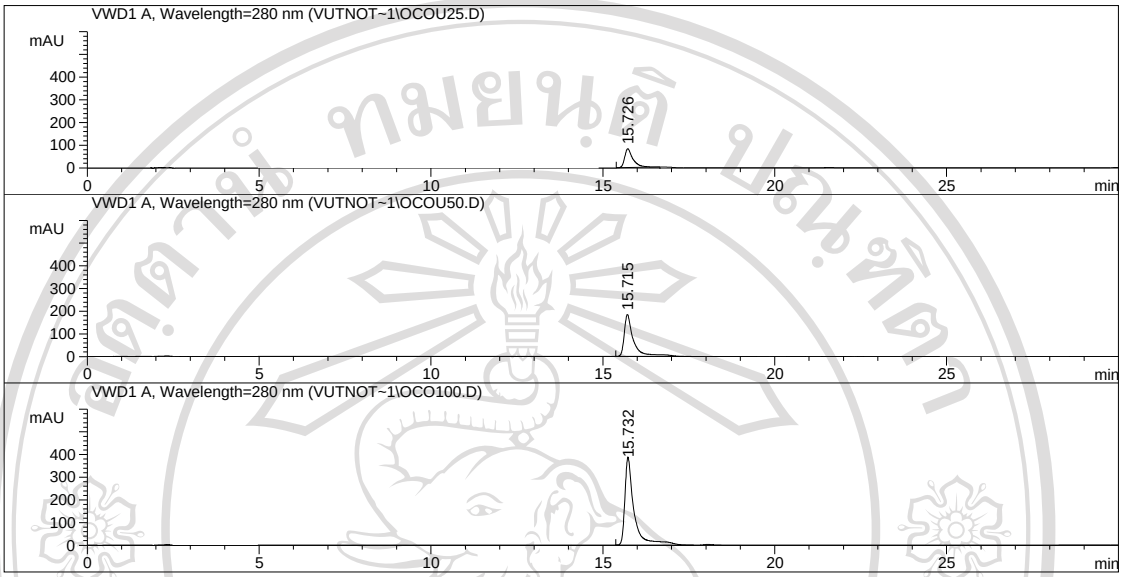


b)

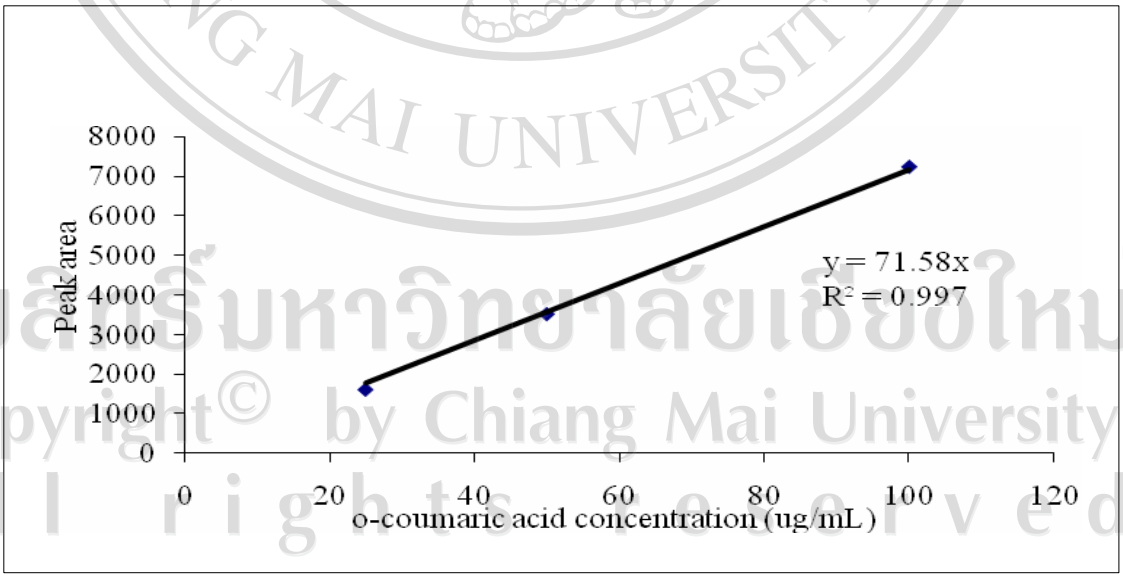


E-6 HPLC chromatograms of (a) m-coumaric acid 25 µg/mL, m-coumaric acid 50 µg/mL and m-coumaric acid 100 µg/mL (b) calibration curve of m-coumaric acid

a)

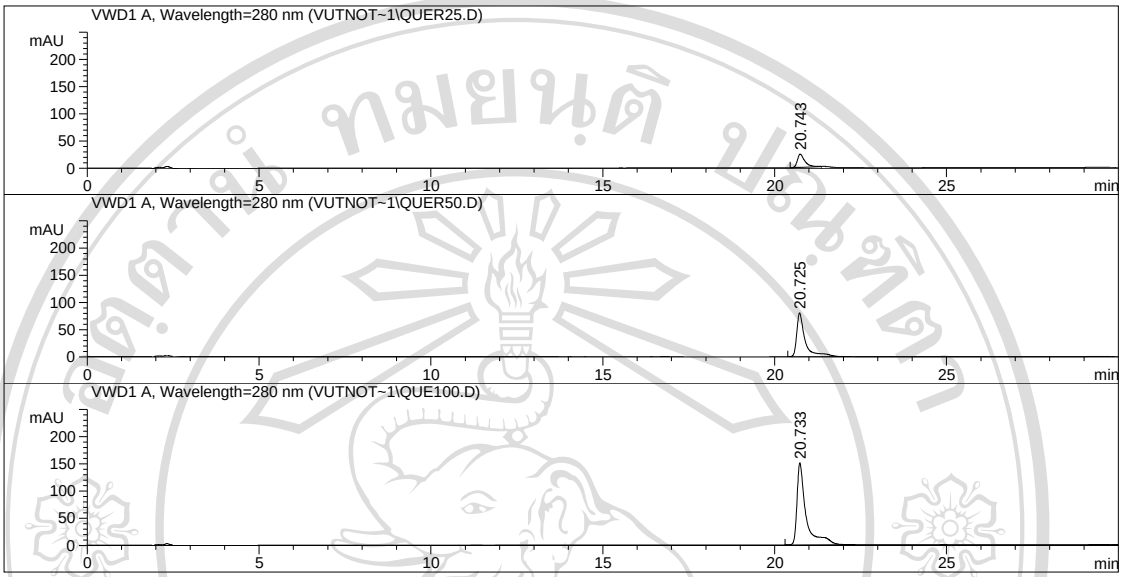


b)

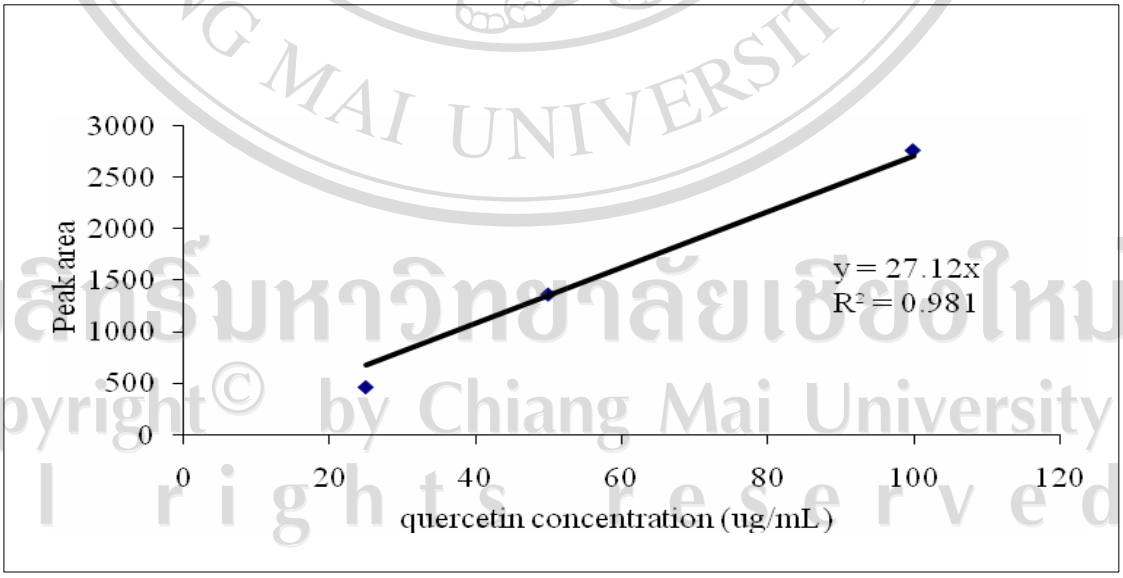


E-7 HPLC chromatograms of (a) o-coumaric acid 25 µg/mL, o-coumaric acid 50 µg/mL and o-coumaric 100 acid µg/mL (b) calibration curve of o-coumaric acid

a)



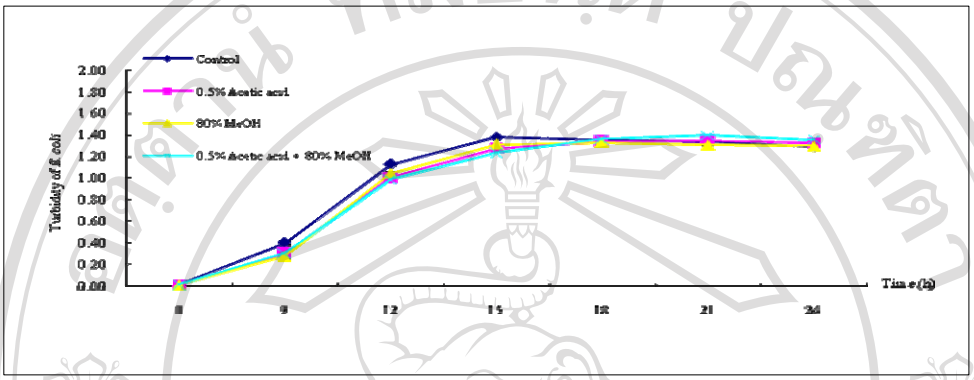
b)



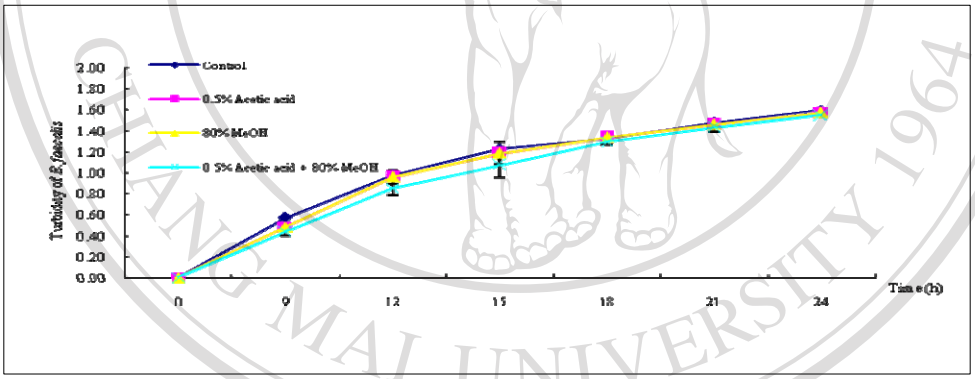
E-8 HPLC chromatograms of (a) quercetin 25 µg/mL, quercetin 50 µg/mL and quercetin 100 µg/mL (b) calibration curve of quercetin

F. Growth inhibition effects of 0.5% acetic acid, 80% methanol and their combination

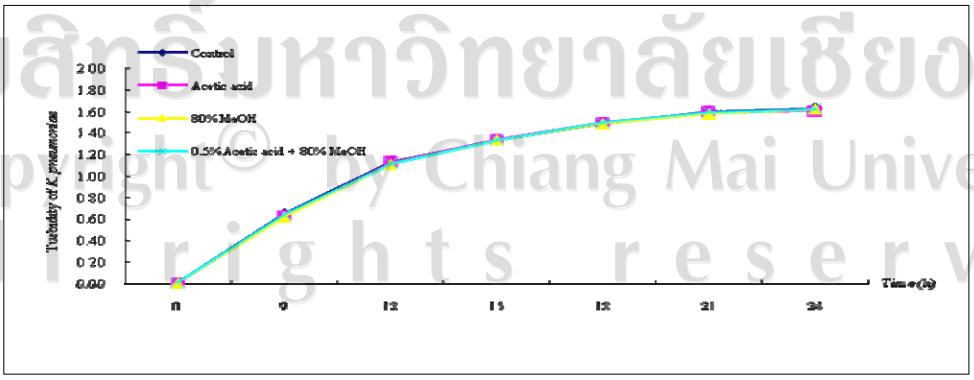
a)



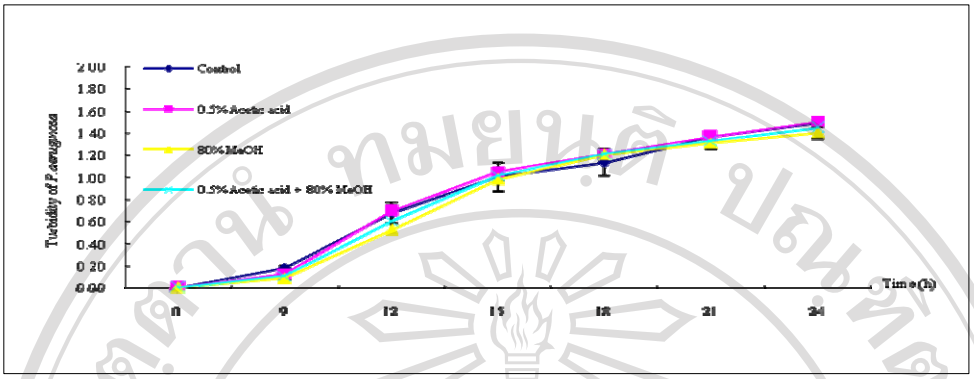
b)



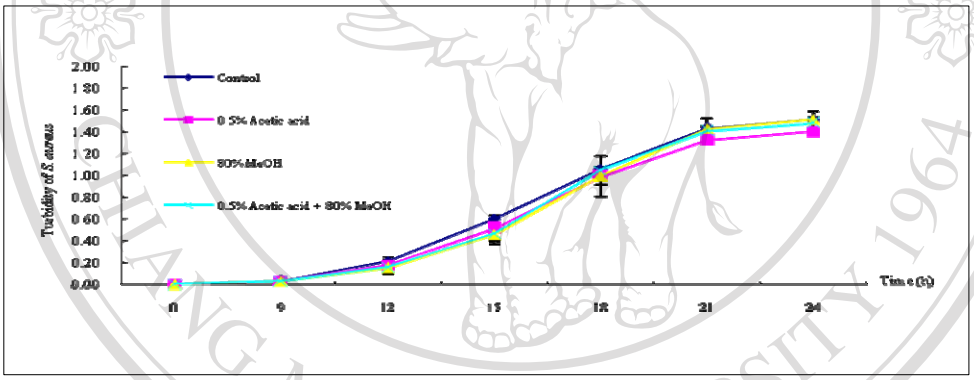
c)



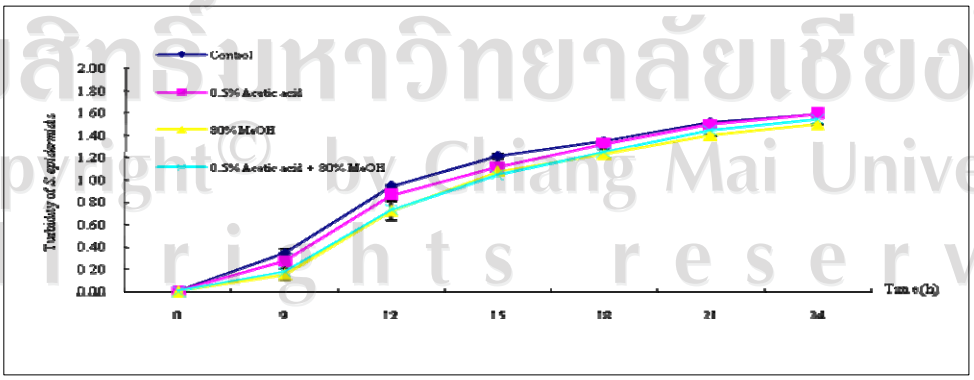
d)



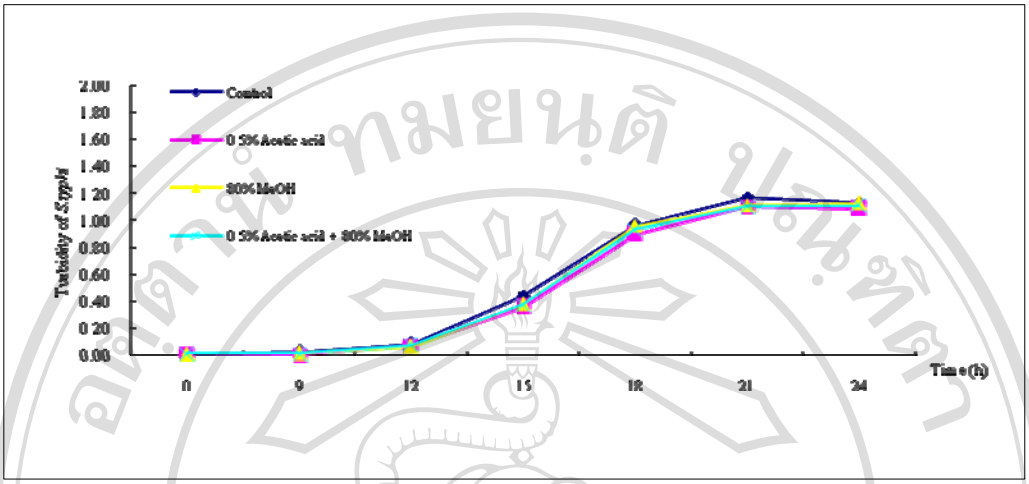
e)



f)

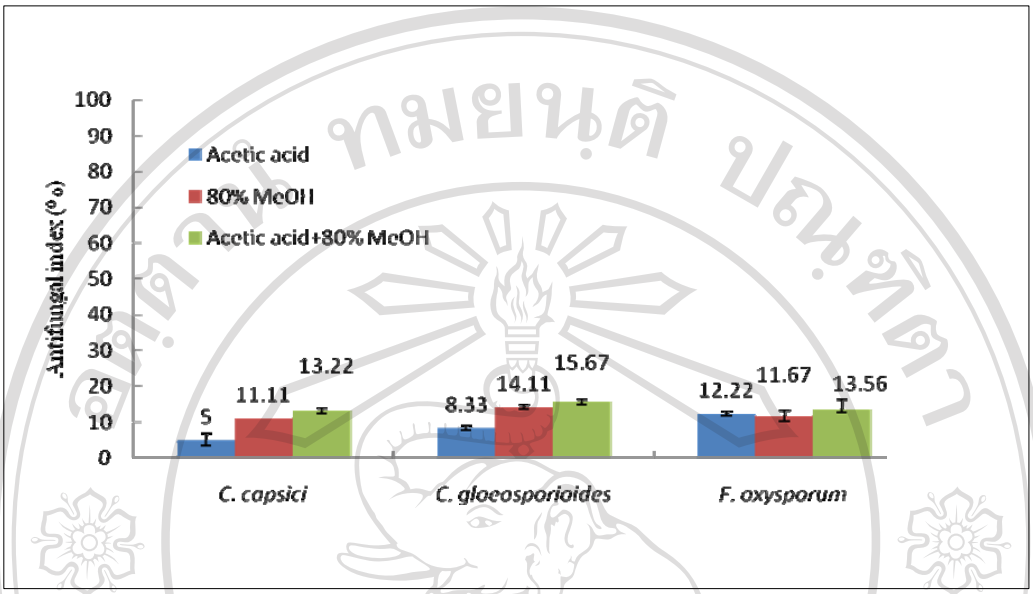


g)



F-1 Growth inhibition effects of 0.5% acetic acid, 80% methanol and their combination on a) *E. coli* b) *E. faecalis* and c) *K. pneumoniae* d) *P. aeruginosa* e) *S. aureus* and f) *S. epidermidis* g) *S. typhi*

G. Antifungal activity of 0.5% acetic acid, 80% methanol and their combination



G-1 Antifungal activity of 0.5% acetic acid, 80% methanol and their combination effect on *C. capsici*, *C. gloeosporioides* and *F. oxysporum*

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Poster presentation

1. Tanreuan K. and Rakariyatham N., 2008, Antioxidant and antibacterial activities of the essential oils and various extracts from *Anethum graveolens* L., Annual Meeting and International Conference of the Thai society for Biotechnology TSB 2008: Biotechnology for Global Care, 14-17 October 2008, Taksila Hotel, Mahasarakham, Thailand.