METHODS DEVELOPMENT OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND CONTINUOUS FLOW SYSTEMS FOR ANTIOXIDANT ANALYSIS

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ABSTRACT

In this research, the high performance liquid chromatography (HPLC) for the simultaneous determination of some antioxidant compounds in banana crude extract including the chromatographic fingerprint analysis, and two continuous flow systems for determination of antioxidant capacity and total phenolic compounds in teas and herbal teas using ABTS and FC assays, respectively, were investigated.

The HPLC method with photodiode array detector (HPLC/DAD) was developed, optimized and validated for the simultaneous determination of gallic acid (GA), gallocatechin (GC), catechin (C), epicatechin (EC) and epigallocatechin gallate (EGCG) in banana crud extracts of raw peel, ripe peel, raw hand stalk, ripe hand stalk, raw bunch stalk and ripe bunch stalk. The chromatographic separation was achieved using a reversed-phase (C18) analytical column and an isocratic elution with a mobile phase of acetonitrile and formic acid. Some parameters were optimized such as the chromatographic separation studies, the detection wavelengths and the ratios of mobile phase. Under optimum conditions, the order of elution was GA, GC, C, EC and EGCG, respectively, with the analysis time per chromatogram of 20 and 50 min for a mixed standard solution and for sample solutions. Linear calibration graphs were in the ranges of 0.25 - 20.0 mg L^{-1} for GA and 0.50 - 30.0 mg L^{-1} for GC, C, EC and EGCG,

respectively. Limit of detections (LOD) were 0.01, 0.07, 0.10, 0.01 and 0.02 mg L^{-1} of GA, GC, C, EC, EGCG, respectively. Limit of quantitations (LOQ) were 0.04, 0.22, 0.32, 0.04 and 0.07 mg L^{-1} of GA, GC, C, EC, EGCG, respectively. Relative standard deviations (RSD) and recoveries in the ranges of 0.2 – 11.1 % and 59 ± 1 – 128 ± 1%, respectively, were obtained for sample analysis. The proposed HPLC system was successfully applied to real samples of banana crude extracts. The proposed HPLC system was provided good resolution, short analysis time, acceptable accuracy and precision. For chromatographic fingerprint analysis, the chromatographic fingerprint patterns of peel, hand stalk and bunch stalk extracts were obtained and six peak markers were found in all part extracts. In peel and hand stalk, six peak markers were at retention times of 4.67 ± 0.17, 5.85 ± 0.21, 10.03 ± 0.18, 11.30 ± 0.06, 14.50 ± 0.13 and 17.55 ± 0.14 min. In bunch stalk, the retention times of six peak markers were 4.67 ± 0.17, 5.85 ± 0.21, 10.03 ± 0.18, 11.30 ± 0.06, 14.50 ± 0.13 and 22.83 ± 0.06 min. The proposed combination of the quantitative and chromatographic fingerprint analyses have been successfully applied for the quantity of some antioxidant compounds and quality profiles in samples of banana crude extract.

Two continuous flow systems with UV/Vis spectrophotometer were developed, optimized and validated for the determination antioxidant capacity using ABTS assay and total phenolic compounds using FC assay in tea and herbal tea samples. The main parameters affecting two systems such as reagent concentrations (e.g. $ABTS^{\bullet+}$, FC and NaOH), volumes of standard/sample, reaction loop lengths, flow rates, and stopped times at reaction loop, were studied. Under optimum conditions, the results of two continuous flow systems were expressed as gallic acid (GA) equivalent. Linear ranges were 0.25 – 2.5 mg L^{-1} of GA for ABTS assay and 2.5 – 15.0 mg L^{-1} of GA for FC assay. LOD were 0.03 and 0.04 mg L^{-1} of GA for ABTS and FC assays, respectively. Sampling throughputs were 21 and 17 injections per hour for ABTS and FC assays, respectively. The proposed two systems of both assays were successfully applied to tea and herbal tea samples and all results were compared with a microplate reader method. In this work, two continuous flow systems could be an alternative methods for screening antioxidant capacity and total phenolic compounds. This two systems offer good accuracy and precision, short analysis time, low consumption of reagent and sample solutions, low waste generation, and cost-effective instrument.

ABBREVIATIONS

ABTS	=	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical
$ABTS^{\bullet+}$	=	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation
$\overline{x}$	=	Arithmetic mean or Average
r^2	=	Square of correlation coefficient
C	=	Catechin
°C	=	Degree celsius
EC	=	Epicatechin
EGCG	=	Epigallocatechin gallate
FC	=	Folin-Ciocalteu's phenol reagent
FIA	=	Flow injection analysis
FTC	=	Flow through cell
GA	=	Gallic acid
GC	=	Gallocatechin
g	=	Gram
HPLC	=	High performance liquid chromatography
h	=	Hour
i.d.	=	Inner diameter
LOD	=	Limit of detection
LOQ	=	Limit of quantitation
µL	=	Microliter
mg	=	Miligram
$mg\ L^{-1}$	=	Milligram per liter
mL	=	Milliliter
$mL\ min^{-1}$	=	Milliliter per minute
min	=	Minute
$mol\ L^{-1}$	=	Mol per liter
nm	=	Nanometer

ABBREVIATIONS (CONT.)

No.	=	Number
% Rec	=	Percentage recovery
%v/v	=	Percentage volume by volume
%w/v	=	Percentage weight by volume
DAD	=	Photo diode array detector
P	=	Peristaltic pump
PTFE	=	Polytetrafluoroethylene
RL	=	Reaction loop
RT	=	Retention time
RRT	=	Relative retention time
RSD	=	Relative standard deviation
s	=	Second
SD	=	Standard deviation
SV	=	Solenoid valve
UV	=	Ultraviolet
Vis	=	Visible
WC	=	Waste coil
W	=	Waste

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CHAPTER I

INTRODUCTION

Rational for the study

An **antioxidant** is a molecule/substance that inhibits the oxidation of other molecules or substances. The oxidation is a chemical reaction that can produce 'free radicals', leading to chain reactions that damage cells. Generally, antioxidants can be divided into two different groups of chemical substances. There are 1) industrial chemicals which are added to products to prevent oxidation, and 2) natural chemicals found in foods and body tissue which are said to have beneficial health effects. Nowadays, an antioxidant is well known as a substance that protects the human body, foods, and non-food commodities (e.g. rubble and plastics) from damage caused by oxidation of harmful molecules or toxic compounds, 'free radicals'. For human body, the oxidation is associated with pathophysiology of human health problems such as carcinogenesis, atherosclerosis and aging. For food and non-food commodities, the oxidation may occur during harvesting, processing, and storage, and is responsible for rancid odors, flavors, or unstable products by the formation of toxic compounds. From the above damage causes, an antioxidant is a useful substance for human body to significantly decrease the adverse effects of reactive species, such as reactive oxygen (ROS) and reactive nitrogen (RNS), on normal physiological functions. Furthermore, an antioxidant is any substance that significantly delays or prevents or greatly retards the oxidation of easily oxidizable nutrients such as 'lipid' or 'fat' in foods [1] and a compound is able to delay, retard or prevent auto-oxidation processes in both of food and non-food products. Thus, antioxidant is employed as preservatives in various products such as in fats, oils, food products, soaps, gasoline, rubber, gum, and other petroleum products.

As the above definitions of antioxidant, it can be categorized into two classes through its mechanisms of 1) primary or chain-breaking antioxidants (mainly acting by ROS/RNS scavenging to break the oxidation chain of lipid radicals involve the sacrificial consumption of antioxidant to produce antioxidant radicals protecting lipid molecules) and 2) secondary or preventative antioxidants (usually acting by transition

metal ion chelation to retard or prevent lipid oxidation) [2]. Moreover, an antioxidant can be classified into three types according to the pathways of its production processes, there are: 1) natural antioxidants (synthesized by various microorganisms, fungi, and even animals, but most often by plants), 2) synthetic antioxidants (produced by human experts by way of synthesis or biosynthesis in the industry), and 3) nature-identical antioxidants (found in foods, but synthesized in the industry) [1]. Among those of antioxidants, the natural antioxidants such as selenium, vitamin A, vitamin C, vitamin E, β-carotene, phenolic acids, and flavonoids are safe and interested in this work. The major sources and approximate contents of natural antioxidants are summarized in Table 1.

Table 1 Major sources and approximate contents of natural antioxidants in some foods, plants and agricultural byproducts [3, 4]

Source	Antioxidant content	Source	Antioxidant content	Source	Antioxidant content
Fruits:		Tomato	68 [b]	Oregano	63 [d]
Apple	296 [a]	**Beverages:**		Rosemary	45 [d]
Banana	90 [a]	Apple juice	339 [c]	Sage	44 [d]
Guava	126 [a]	Orange juice	755 [c]	**Grains:**	
Litchi	4 [a]	Green tea	66 to 106 [c]	Bean	0.8 [d]
Papaya	58 [a]	Instant coffee	146 to 151 [c]	Pistachio	2 [d]
Blueberry	270 to 930 [a]	Rose wine	1304 [c]	Sunflower seed	6 [d]
Vegetables:		Red wine	1593 to 1637 [c]	**Agricultural by-products:**	
Broccoli	102 [a]	**Herbs:**		Almond hull	43 [e]
Carrot	56 [a]	Cinnamon	77 [d]	Apple peel	169 to 2299 [e]
Cucumber	20 [a]	Ginger	20 [d]	Dried apple pomace	318 to 861 [e]
Mint	400 [a]	Mint leave	116 [d]		

[a] mg gallic acid equivalents/100 g fresh weight, [b] mg catechin equivalents/100 g fresh weight, [c] mg gallic acid equivalents/L, [d] mmol/100 g, [e] mg/100 g fresh weight

Natural phenolic antioxidants are the most compounds (more than 8,000 compounds) that have been reported in plants, especially in the best sources of fruits, vegetables and herbs. The examples of phenolic antioxidants are identified and presented in some plants as following. Phenolics in apple varieties are normally found hydroxycinnamic acid (e.g. chlorogenic acid), flavanols (e.g. catechin and epicatechin),

flavonols (e.g. rutin and isoquercitrin) and chalcones (e.g. phlorizidin) [5]. Grape berries and their skins commonly contain phenolic acids (e.g. caftaric acid and coutaric acid), flavonols (e.g. guercetin 3-glucuronide and myricetin 3-glucurin) and flavanonols (e.g. astilbin and engeletin) [5]. Blueberries are rich sources of phenolic acids (e.g. gallic acid and caffeic acid), flavanols (e.g. catechins), anthocyanins (e.g. cyaniding and delphinidin) and flavonols (e.g. quercetin and kaempferol) [5]. Banana and its wastes serve as a good source of phenolic acids (e.g. gallic acid), flavanols (e.g. catechin and epicatechin) anthocyanins (e.g delphinidin and cyanidin) and tannin [6]. Onions are rich sources of flavonols (e.g. quercetin, isorhamnetin, myricetin and kaempferol) and anthocyanins (e.g. peonidin 3-glucoside and cyaniding 3-glucoside) [5]. Spinach contains flavonols (e.g. patuletin, jaceidin and spinacetin) [5]. Teas consist of flavonls (e.g catechins, thearubigenes and theaflavins) [7]. Gingers contain phenolic acids (e.g. caffeic acid and chlorogenic acid), flavonols (e.g. kaempferol and quercetin) and anthocyanin (e.g. delphinidin) [8]. Sage is rich sources of phenolic acids (e.g caffeic acid, carnosic acid and ferulic acid), flavanols (e.g catechin) and tannin [8]. From those natural phenolic compounds, it can be classified in term of the basic carbon skeleton as shown in Table 2.

Table 2 The classification of natural phenolic compounds under the basic carbon skeleton [9]

Basic carbon skeleton	Classes	Natural phenolic compounds
C_6	Simple phenolics	Catechol, Resorcinol
C_6-C_1	Phenolic acids	Salicylic acid
C_6-C_2	Phenylacetic acids	p-Hydroxyphenylacetic acid
C_6-C_3	Cinnamic acids	Caffeic acid, Ferulic acid
	Phenylpropenes	Eugenol, Myristicin
	Coumarins	Aeculetin, Scopolin
	Chromones	Eugenin
C_6-C_4	Naphthoquinoes	Juglone
C_6-C_1-C_6	Xanthones	Mangostin, Mangiferin
C_6-C_2-C_6	Stillbenes	Resveatrol
	Anthraquinones	Emodin

Table 2 (cont.)

Basic carbon skeleton	Classes	Natural phenolic compounds
C_6-C_3-C_6	Flavonoids:	
	Flavones	Sinensetin, Nobiletin, Tangeretin
	Flavonols	Quercetin, Kaempferol
	Flavonol glycosides	Rutin
	Flavanonols	Dihydroquercetin
	Flavanones	Hesperitin, Naringenin
	Flavanone glycosides	Hesperidin, Neohesperidin, Narirutin
	Anthocyanins	Naringin, Delphinidin, Petunidin
	Flavanols	(+)-Catechin, (-)-Epicatechin
	Chalcones	Arbutin, Chalconaringenin
$(C_6$-$C_3)_2$	Lignins	Pinoresinol
$(C_6$-C_3-$C_6)_2$	Biflavonoids	Agathiflavone

Several analytical methods have been used for identifying and quantifying the natural phenolic antioxidants in varieties of fruit, vegetable, herb, tea and also herbal tea samples in order to investigate the fingerprint of phenolic compounds and evaluate for phenolic contents during its harvest, production process and packaging. These methods include capillary electrophoresis, high performance liquid chromatography (HPLC) and gas chromatography [5, 10]. According to review papers, the HPLC method has been widely employed because of its high separation capacity [11] and reliable technique [9]. The applications of HPLC have been reported in many purposes for phenolic compound analysis in various samples of bananas and teas, such as the analysis of tannins in green banana flesh [12], flavonol compounds in banana peel (*Musa cavendish*) [13] and catechins in food [14, 15].

Because of the diversity of the natural antioxidants, it is difficult to analyze antioxidant compounds from foods, plants and biological matrices via analytical methods as mention above. Thus, the measurement of antioxidant activity and capacity from directly plant and food extracts is desirable. The antioxidant capacity/activity level of foods is mostly concerned with methods of measuring chain-breaking or preventive antioxidant ability for the meaningful comparison of the antioxidant content of foodstuffs and for the diagnosis and treatment of oxidative stress-associated diseases in clinical biochemistry [16]. The basis of the chemical reactions involved are hydrogen

atom transfer (HAT)-based assays, electron transfer (ET)-based assays and mixed-mode (ET and HAT-based) assays [2, 16]. The HAT-based assays measure the capability of an antioxidant to quench free radicals (mainly peroxyl radicals or ROO$^{\bullet}$) by H atom donation. The HAT mechanisms in which the hydrogen atom/hydrogen radical (H$^{\bullet}$) of an antioxidants (AH) or a phenolic compounds (ArOH) is transferred to an ROO$^{\bullet}$ radical as an equation bellow, and the antioxidant radicals (A$^{\bullet}$) or aryloxy radicals (ArO$^{\bullet}$) formed from the reaction of antioxidant phenol with peroxyl radical is usually stabilized by resonance.

$$ROO^{\bullet} + AH/ArOH \longrightarrow ROOH + A^{\bullet}/ArO^{\bullet} \quad (1)$$

Generally, the HAT-based assays are such as oxygen radical absorbance capacity (ORAC) assay, total peroxyl radical trapping antioxidant parameter (TRAP) assay using R-phycoerythrin as the fluorescent probe, and crocin bleaching assay using 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) as the radical generator. The electron transfer (ET)-based assays are based on the detection of an antioxidant ability to transfer one electron to reduce any biologically radicals as following reactions.

$$ROO^{\bullet} + AH/ArOH \longrightarrow ROO^{-} + AH^{\bullet+}/ArOH^{\bullet+} \quad (2)$$

$$AH^{\bullet+}/ArOH^{\bullet+} + H_2O \longleftrightarrow A^{\bullet}/ArO^{\bullet} + H_3O^{+} \quad (3)$$

$$ROO^{-} + H_3O^{+} \longleftrightarrow ROOH + H_2O \quad (4)$$

The ET-based assays include Folin-Ciocalteu (FC) assay, ferric reducing antioxidant power (FRAP) assay, cupric reducing antioxidant capacity (CUPRAC) assay and ferricyanide (Hexacynoferrate (III))-Prussian blue assay. The mixed-mode (HAT- and ET-based) assays are generally based on the scavenging of a stable radical by transfer proton coupled electron of antioxidants. The mixed-mode assays include 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, 2,2–diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, N,N-dimethyl-ρ-phenylenediamine dihydrochloride (DMPD) radical scavenging assay, reactive oxygen species/reactive nitrogen species (ROS/RNS) scavenging methods, and cellular antioxidant activity (CAA) assays.

Generally, the assays of DPPH, ABTS, FRAP and FC are frequently used for especially the analysis of food and natural product samples. The ABTS and FC assays are normally determined by the ultraviolet/visible (UV/Vis) spectrophotometry [17, 18, 19, 20] and electrochemistry [21, 22]. According to reviewed papers, some of these techniques give different disadvantages such as none automated techniques, tedious in operation, high consumption of reagent and sample solutions, high waste generation and long analysis time. To compromise for these reasons, the continuous flow system is required to determine antioxidant capacity such as flow injection analysis (FIA) using ABTS [23, 24] and FC [25, 26] assays and sequential injection analysis (SIA) using ABTS [27, 28] and FC [29, 30] assays.

In this work, the methods of HPLC and continuous flow systems will be developed for the analysis of some antioxidant compounds and antioxidant capacities using ABTS and FC assays, respectively, in plant samples such as banana or its wastes (e.g. peel, hand stalk and bunch stalk), teas and herbal teas.

Objectives of the study

1. To develop, optimize the conditions, and validate a HPLC method for the determination of some antioxidant compounds in banana wastes extract (e.g. peel, hand stalk and bunch stalk) and chromatographic fingerprint analysis.

2. To develop, optimize the conditions, and validate the continuous flow system using ABTS assay for the determination of antioxidant capacity in teas and herbal teas.

3. To develop, optimize the conditions, and validate the continuous flow system using FC assay for the determination of total phenolic compounds in teas and herbal teas.

Scopes of the study

Some natural antioxidant compounds (e.g. gallic acid, gallocatechin, catechin, epicatechin and epigallocatechin gallate) in bananas waste extracts of peel, hand stalk and bunch stalk will be analyzed by the method development of HPLC including the chromatographic fingerprint analysis. Some antioxidant capacities in teas and herbal teas using ABTS and FC assays will be analyzed by the development of continuous flow systems. The proposed HPLC method will offer good resolution, short analysis time,

acceptable accuracy and precision, and obtainable chromatographic fingerprint patterns of the samples. And the proposed continuous flow systems will provide easy operation, automatic or semi-automatic feature, low sample and reagent consumption, low waste collection, short analysis time and cost effective instruments.

Expected benefits

1. Achieve the HPLC method for the determination of gallic acid, gallocatechin, catechin, epicatechin, and epigallocatechin gallate in banana waste extracts of peel, hand stalk and bunch stalk including chromatographic fingerprint pattern of the samples.

2. Achieve the continuous flow system using ABTS assay for the determination of antioxidant capacity in teas and herbal teas.

3. Achieve the continuous flow system using FC assay for the determination of total phenolic compounds in teas and herbal teas.

4. Present the results in the national and/or international academic conference by poster and/or oral presentation or published the academic paper.

CHAPTER II

REVIEW OF RELATED LITERATURE AND RESEARCH

Banana waste

A banana waste is some parts of banana residues that are thrown away by farmers, consumers, markets and also supermarkets. These wastes are such as peel, hand stalk, bunch stalk, pseudostem, midrib, pulp residue, leaf, old leaf, root, rachis and bract (shown in Figure 1). A huge mass of banana residues is still dumped as waste and is a major problem of the waste management. Banana wastes are reported to utilize in several ways such as pharmacology, animal feed and industrial product. For pharmacology, peel and pulp waste contain serotonin and dopamine hormones that are used for antifungal and antibiotic [31]. Root is used to treat digestive disorders. For animal feed, pulp residue, pseudostem and bract are raw materials for pig, fish, and chicken feeds. For industrial product, leaf, pseudostem, bunch stalk and hand stalk containing abundant fiber are transformed to bio-products such as yarn, paper, gummy bags and door mats [32, 33]. Therefore, this work interests to increase the value of banana wastes especially peel, hand stalk and bunch stalk residues for investigation antioxidant compounds.

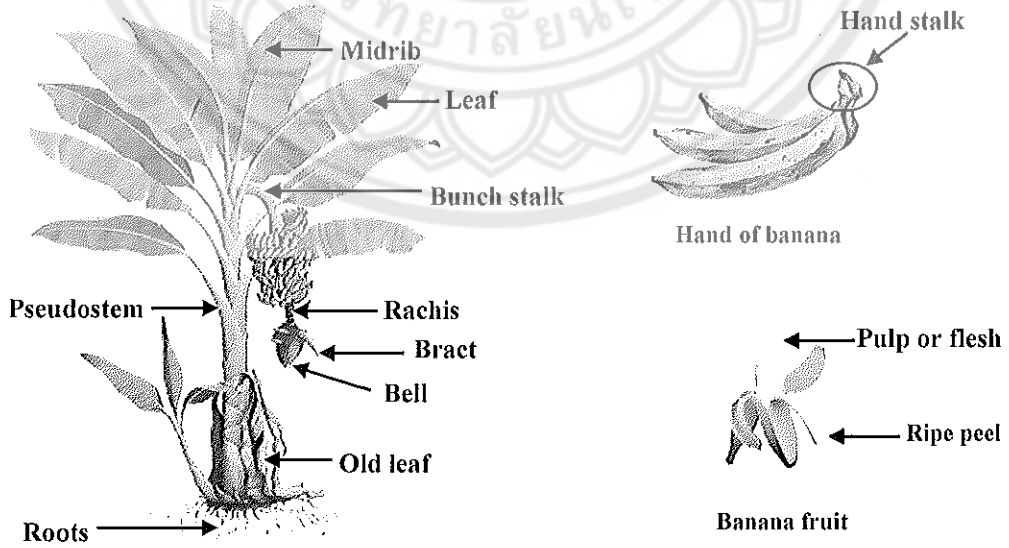


Figure 1 The banana plant (adapted from reference [34, 35, 36])

Antioxidant compounds found in banana wastes have been studied and presented by several researchers. Banana peel is rich source of saponin (approximately 24 mg per g of fresh weight [37, 38]) and phenolic compounds (about 11 to 58 mg per g of dry weight [39]). Banana bell contains rich anthocyanin around 32 mg per 100 g of fresh weight [40]. Banana pseudostem is found some phenolic acids and flavonoid (approximately 2 to 51 and 5 μg per mg of extract, respectively [41]). And banana pulp waste is found vitamin A and ascorbic acid (approximately 8 to 12 μg per 100 g and 5 to 13 mg per 100 g of fresh weight, respectively [42]), carotenoids (around 130 to 9400 μg per 100 g of fresh weight [43]) and phenolic compounds (about 0.03 to 10 mg per 100 g of fresh weight [44]). In this work, phenolic compounds of gallic acid, gallocatechin, catechin, epicatechin and epigallocatechin gallate (chemical information of these compounds shown in Table 3) are interested because these compounds are generally found in fruit and teas. Gallic acid has cytotoxic and antioxidant activities such as anti-inflammatory, antitumor, antimutagenic and anticarcinogenic agents [45, 46, 47]. Catechin groups have been associated as being anti-inflammatory, antioxidant, anticarcinogenic, antiobesity, antitumorigenic and antiallergic [48] and have been found in banana peel.

Table 3 Chemical information of antioxidant compounds used in this work [49]

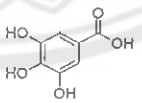
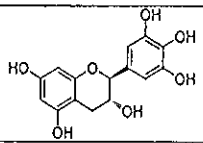
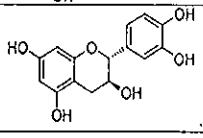
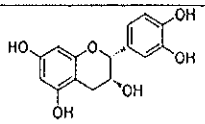
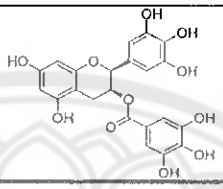
Antioxidant compounds (abbreviation)	IUPAC name	Structural formula	Molecular formula (molar mass; g mol^{-1})	Solubility (pK_a)
Gallic acid (GA)	3,4,5-trihydroxyben-zoic acid		$C_7H_6O_5$ (170.12)	water, alcohol, ether and acetone (4.40)
(-)-Gallocate-chin (GC)	(2S,3R)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-1(2*H*) benzo pyran-3,5,7-triol		$C_{15}H_{14}O_7$ (306.27)	water and alcohol (8.41)
(+)-Catechin (C)	(2R,3S)-2-(3,4-dihydroxy phenyl)-3,4-dihydro-1(2*H*)-benzo pyran-3,5,7-triol		$C_{15}H_{14}O_6$ (290.27)	water and alcohol (8.64)

Table 3 (cont.)

Antioxidant compounds (abbreviation)	IUPAC name	Structural formula	Molecular formula (molar mass; g mol^{-1})	Solubility (pK_a)
(-)-Epicatechin (EC)	(2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-1(2*H*)-benzo pyran-3,5,7-triol		$C_{15}H_{14}O_6$ (290.27)	water and alcohol (8.72)
(-)-Epigallo-catechin gallate (EGCG)	(−)-cis-2-(3,4,5-trihydroxyphenyl) -3,4-dihydro-1(2*H*)-benzopyran-3,5,7-triol 3-gallate		$C_{22}H_{18}O_{11}$ (458.37)	water and ethanol (7.68)

There are several **analytical methods** for identification, quantification and fingerprint analysis of antioxidants as phenolic compounds in herbs, fruits, vegetables and beverage such as capillary electrophoresis (EC) [10, 50, 51, 52], gas chromatography (GC) [53, 54], and high performance liquid chromatography (HPLC) [44, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65]. Among these methods, HPLC is the most generally used for the determination of phenolic compounds with various detectors such as ultraviolet (UV) [55, 56, 57, 58, 59], photo diode array (DAD) [44, 61, 62, 63], fluorescence [60] and UV or DAD couple with mass spectrometer (UV/MS or DAD/MS) [12, 13, 64, 66] detectors. In addition to clean sample up and sample purification, a sample separation by extraction technique is necessary for phenolic compounds analysis by HPLC. According to review papers, many extraction techniques prior to HPLC are applied in various samples such as liquid liquid extraction (LLE) for green tea to determine epigallocatehin gallate, epicatechin, catechin and caffeine [59] and solid phase extraction (SPE) for alcohol-free beer to analyze phenolic acids, flavonols and flavanols [58]. The HPLC/UV and HPLC/DAD with extraction techniques for identification and quantification of phenolic compounds in banana and other samples are summarized in Table 4. Furthermore, the HPLC methods with various detectors and samples have been also presented for HPLC fingerprint analysis. These fingerprint analyses are for example 1) Britto et al. using UV detector to achieve five peak markers for quantification and identification of *Vitex negundo* L. [67], 2) Ma et al. using DAD detector to gain ten peak markers for authentication of Hawk-tea [68]

and 3) Sirikatitham et al. using also DAD detector to achieve three peak markers for identification and assessment of *Dioscorea membranacea* Pierre [69].

Table 4 Some relevant HPLC/UV and HPLC/DAD methods used for the determination of antioxidant compounds in banana and other samples

Methods	Details	Year [Ref.]
HPLC/DAD	**Sample:** Sea buckthorn extract **Analytes:** Catechin (C), rutin (RU), quercetin (QU) kaempferol (KA) and isorhamnetin (IS) **Extraction technique:** LLE (extraction solvent 80% ethanol) **HPLC conditions:** Column = HIQ SIL C18V, MP = 40:15:45 (v:v:v) of methanol:acetonitrile:1.0 % acetic acid, F = 1.0 mL min^{-1}, and λ = 257 nm for RU, 279 nm for C and 368 nm for QU, KA and IS **Analytical characteristics:** LR = 0.011 – 0.520 mg C mL^{-1}, 0.007 – 0.500 mg RU mL^{-1}, 0.019 – 0.280 mg QU mL^{-1}, 0.010 – 0.440 mg KA mL^{-1} and 0.008 – 0.400 mg IS mL^{-1}, % RSD = 0.2 – 0.8, LOD = 0.00079 – 0.00290 mg mL^{-1} and % Rec = 97 – 99 of all compound	2006 [63]
HPLC/UV	**Sample:** Green tea **Analytes:** Epigallocatechin gallate (EGCG), epicatechin (EC), catechin (C) and caffeine (CAF) **Extraction technique:** LLE (extraction solvent 89:6:1:3:1 (v:v:v:v:v) of water:acetonitrile: methanol:ethyl acetate:glacial acetic acid) **HPLC conditions:** Column = LiChrosorb RP-18, MP = 89:6:1:3:1 (v:v:v:v:v) of water:acetonitrile:methanol:ethyl acetate:glacial acetic, F = 0.7 mL min^{-1} and λ = 280 nm of all analytes **Analytical characteristics:** LR = 60 – 300 µg mL^{-1} of all analytes and %RSD = 0.3 – 2.6	2006 [59]
HPLC/DAD	**Sample:** Banana pulp waste **Analytes:** Gallic acid (GA) and catechin (C) **Extraction technique:** Solid phase extraction (extraction solvent 50% methanol) **HPLC conditions:** Column = Waters Nova-Pack C18, MP = 88:10:2 (v:v:v) ofwater:methanol:acetic acid, F = 1.0 mL min^{-1} and λ = 280 nm **Analytical characteristics:** %RSD = 0.44 – 3.77 and 0.12 – 2.51 and % Rec = 49.6±1.5 and 84.3±2.2 for C and GA	2003 [44]

Table 4 (cont.)

Methods	Details	Year [Ref.]
HPLC/UV	**Samples:** *Michelia alba* extract, *Caesalpinia pulcherrima* and *Nelumbo nucifera* (flower) **Analytes:** Gallic acid (GA), catechin (C), rutin (RU), ellagic acid (EA) and quercetin (QU) **Extraction technique:** LLE (extraction solvent 95% ethanol) **HPLC conditions:** Column = Luna C18, MP = 25:1 (v:v) of water:acetic acid (eluent A) and methanol (eluent B), F = 1.0 mL min^{-1} and λ = 280 nm of all analytes **Analytical characteristics:** LR = 2.62 – 21 μg GA mL^{-1}, 10.85 – 86.80 μg C mL^{-1}), 10 – 80 μg RU mL^{-1}, 10.05 – 80.40 μg EA mL^{-1} and 10.05 – 80.40 μg QU mL^{-1}, % RSD = 1.0 – 1.8, LOD = 0.37 – 1.32 μg mL^{-1} and % Rec = 96 - 102	2002 [57]
HPLC/UV	**Samples:** Fruits and legumes **Analytes:** (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) **Extraction technique:** LLE (extraction solvent 90% methanol) **HPLC conditions:** Column = Inertsil ODS-2, MP = 5% acetonitrile and 0.025 mol L^{-1} phosphate buffer pH 2.4, F = 1.0 mL min^{-1}, and λ = 270 nm of all analytes **Analytical characteristics:** LR = 2 – 10 μg mL^{-1} for C and EC, 5 – 15 μg mL^{-1} for ECG and EGCG and 10 – 30 μg EGC mL^{-1}, % RSD = 1.3 – 9.0 %, LOD = 0.03 – 0.13 μg mL^{-1} and % Rec = 92 – 105	1998 [56]

LLE – Liquid – liquid extraction, MP – Mobile phase, F – Flow rate, λ – Wavelength detection, LR – Linear range, %RSD – Percentage relative standard deviation, LOD – Limit of detection and % Rec – Percentage recoveries

Teas and Herbal teas

In Thailand, the meaning of tea and herbal tea is defined by the Food and Drug Administration department, Ministry of Public Health (assigned in No. 196 B.E. 2543 (2000) [70] and No. 280 B.E. 2547 (2004) [71]) as following. **Tea** is an aromatic beverage made from young dried leaves, crowns and branches of tea in *Camellia* Family in hot water and also made from camellia leaves containing herb and other plants less than 10%. **Herbal tea** is an aromatic beverage made from infusion of herbs, fruits or

other plant materials (containing more than 90%) in hot water. Drinking tea and herbal tea are became popular in Thai people over centuries. Because of the benefit of promoting health and preventing many diseases such as cancer, arthritis, insomnia and diabetes are presented. Tea products might consist of fruit, vegetable and herb containing high level of antioxidant activities/capacities. These levels of the product indicated prevention/inhibit ability of diseases caused by free radicals.

There are many herb to make herbal tea such as ginger, sage, peppermint, cinnamon, berry fruits and other herbs. The herb has been reported the contents of antioxidant capacity and total phenolic compounds as following. Green teas, Oolongs, black teas are found contents of antioxidant capacity around 1236 to 3307, 1222 to 2906 and 212 to 1578 mmol L^{-1} of trolox per kg dry weight and total phenolic compounds approximately 422 to 818, 412 to 722 and 125 to 530 mmol L^{-1} of gallic acid per kg weight, respectively [72]. Sage, peppermint, thyme, absinthium, roselle, relax teas have total phenolic compounds around 330, 3,750, 1,510, 570, 170 and 860 mg catechin equivalent per L of tea, respectively [73]. In this work, the determination of antioxidant capacity and total phenolic compounds using ABTS and FC assays in tea and herbal tea are interested. The ABTS assay are rapid reaction, run over a wide range of pH and $ABTS^{\bullet+}$, and soluble in both aqueous and ogainc solvent [16]. For FC assay is commercially available and the procedure is preferably standardized. FC is a commonly and routinely accepted assay and low interference at long detection wavelength (730 nm) [74].

There are popular **analytical methods** for the determination antioxidant capacity and total phenolic compounds using ABTS and FC assays that are UV/Vis spectrophotometry [17, 18, 65, 75, 76, 77] (see in Table 5) and electrochemistry [22, 78]. According to review papers, a popular technique of sample separation for the determination antioxidant activity/capacity analysis in tea and herbal tea is liquid extraction. Nevertheless, a lot of sample solutions, long analysis time and high waste generate are concerned for antioxidant capacity and total phenolic compounds analyses by UV/Vis. Therefore to compromise these reasons, the continuous flow system is required to determine antioxidant capacities in teas and herbal teas. The related literatures on the determination of antioxidant capacity using ABTS and FC assays are summarized in Table 5.

Table 5 UV/Vis spectrophotometry and continuous flow system for the determination of antioxidant capacity using ABTS assay and total phenolic compounds using FC assay in tea and herbal tea

Methods	Details	Year [Ref.]
UV/Vis spectrophotometry	**Sample:** Herbals and green teas **Analyte:** Antioxidant capacity and total phenolic compounds **ABTS conditions:** Std = trolox, $ABTS^{\bullet+}$ = 7.0 mmol L^{-1} ABTS + 2.45 mmol L^{-1} $K_2S_2O_8$ + 80% v/v ethanol, reaction time = 6 min, and λ = 734 nm **FC conditions:** Std = gallic acid (GA), FC = 5 – 100 µg GA mL^{-1} + 0.5 N FC reagent + 75.0 g L^{-1} Na_2CO_3, reaction time = 120 min, and λ = 750 nm **Analytical characteristic:** %RSD = 11.2 – 154.1 and 7.49 – 111.5 for ABTS and FC assays	2016 [79]
UV/Vis spectrophotometry	**Sample:** Herbal teas **Analyte:** Antioxidant capacity and total phenolic compounds **ABTS conditions:** Std = ascorbic acid, $ABTS^{\bullet+}$ = 7.4 mmol L^{-1} ABTS + 2.45 mmol L^{-1} $K_2S_2O_8$ + ethanol, reaction time = 60 min, and λ = 734 nm **FC conditions:** Std = gallic acid (GA), FC = GA + 1:10, FC reagent:water + 2 % w/v Na_2CO_3, reaction time = 30 min, and λ = 750 nm **Analytical characteristic:** %RSD = 0.21 – 16.21 and 0.28 – 8.50 for ABTS and FC assays	2013 [65]
UV/Vis spectrophotometry	**Sample:** Herbal teas **Analyte:** Total phenolic compounds **FC conditions:** Std = gallic acid (GA), FC = 31.1 – 500 µg GA mL^{-1} + 1:10, FC reagent:water + 20 % w/v Na_2CO_3, reaction time = 30 min, and λ = 765 nm **Analytical characteristic:** %RSD = 0.83 – 2.88	2012 [75]
UV/Vis spectrophotometry	**Sample:** Herbal teas **Analyte:** Total phenolic compounds **FC conditions:** Std = tannic acid (TA), FC = GA + 1:10, FC reagent:water + 10 % w/v Na_2CO_3, reaction time = 60 min, and λ = 760 nm **Analytical characteristic:** % RSD = 7.10 – 33.33	2011 [77]

Table 5 (cont.)

Methods	Details	Year [Ref.]
FIA/UV	**Sample:** Teas **Analyte:** Total phenolic compounds **FC conditions:** Std = gallic acid (GA), FC = GA + 0.2 mol L^{-1} FC reagent + 75 g L^{-1} Na_2CO_3, reaction time = 0 min, and λ = 765 nm **Analytical characteristic:** LR = 0.5 – 100 mg L^{-1}, LOD = 0.0231 mg L^{-1}, % RSD = 0.08 – 0.45 and sample throughput = 32 samples h^{-1}	2009 [26]
MSFIA/ UV	Sample: Herbal and tea infusions, wine, juice, and beer Analyte: Antioxidant capacity and total phenolic compounds **ABTS conditions:** Std = trolox, $ABTS^{\bullet+}$ = 18 mmol L^{-1} ABTS + 20 mmol L^{-1} H_2O_2 + 3.2x10^{-6} units L^{-1} HRP + acetate buffer pH 4.6, reaction time = 4:55 min and λ = 734 nm FC condition: Std = gallic acid (GA), FC = GA + 1:10, FC reagent:water + 0.25 mol L^{-1} NaOH, reaction time = 4:10 min, and λ = 750 nm Analytical characteristics: LR = 0.020 – 0.20 mmolL^{-1} of trolox and 5.0 – 75 mg $L^{-1}$of GA, LOD = 0.008 mmolL^{-1} and 3 mg L^{-1}, % RSD = 23.79 – 52.61 and 21.7 – 61.37 for ABTS and FC assays and sample throughput = 24 samples h^{-1} of both assays	2007 [80]
FIA/UV	**Sample:** Teas, wines, beers, soft drinks, fruit juices **Analyte:** Total phenolic compounds **FC conditions:** Std = gallic acid (GA), FC = GA + 1:10, FC reagent:water + 0.25 mol L^{-1} NaOH, reaction time = 4 min, and λ = 750 nm **Analytical characteristics:** LR = 2.5 – 40.0 mg L^{-1}, LOD[c] = 0.6 mg L^{-1}, % RSD = 0.08 – 0.45 and sample throughput = 12 samples h^{-1}	2006 [81]
SIA/UV	Sample: Black and green teas, fruit juices, beer, milk, and yoghurt Analyte: Antioxidant capacity **ABTS conditions:** Std = ascorbic acid, $ABTS^{\bullet+}$ = 7 mmol L^{-1} ABTS + 2.45 mmol L^{-1} $K_2S_2O_8$ + water, reaction time = 4 min, and λ = 734 nm **Analytical characteristics:** LR = 5 - 20 µmol L^{-1} and % RSD = 0.15 - 24.32	2005 [27]

Table 5 (cont.)

Methods	Details	Year [Ref.]
FIA/UV	**Sample:** Black tea, coffee, juices, cola, lemon ice tea, and beer	2003
	Analyte: Antioxidant capacity	[24]
	ABTS conditions: Std = trolox, $ABTS^{\bullet+}$ = 7 mmol L^{-1} ABTS + 2.45 mmol L^{-1}	
	$K_2S_2O_8$ + ethanol, reaction time = 1 min, and λ = 734 nm	
	Analytical characteristics: LR = 10 – 300 µmol L^{-1}, LOD = 4.14 µmol L^{-1},	
	% RSD = 0.29 – 11.11, and sample throughput = 30 samples h^{-1}	

Std – Standard solution, Reaction time – Time of reaction between $ABTS^{\bullet+}$ and antioxidant and Folin–Ciocalteu reagent, antioxidant and alkaline solution, respectively, λ – Detection wavelength, %RSD – Relative standard deviation, LR – Linear range, LOD – limit of detection, MSFIA – Multisyringe flow injection analysis and HRP – Horseradish peroxidase

High performance liquid chromatography

1. General and principle

High performance liquid chromatography (HPLC) [82, 83, 84, 85, 86] is a technique in analytical chemistry used to separate, identify and quantify each component (or solute) in a mixture solution which relies on the distribution of each liquid-component molecule by a high-pressure pump between two phases: a stationary phase and a mobile liquid phase. The stationary phase is a solid adsorbent material (e.g. silica, alumina, polymer or various liquid coated onto a solid support) filled into a small column while the pressurized liquid of the typical mixture solvents (e.g. water, acetonitrile and/or methanol) is referred to as a "mobile phase". Therefore, each component in the sample interacts slightly different with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. In general, three primary characteristics of chemical compounds can be used to create HPLC separations. They are a polarity, an electrical charge and a molecular size. For the polarity characteristic, the HPLC technique can be divided into two primary separation modes: normal phase and reversed phase chromatography. A normal phase HPLC is using a polar stationary phase with a much less polar (or non-polar) mobile phase while a reversed phase HPLC describes the chromatography mode using a non-polar (hydrophobic) stationary phase and a polar mobile phase. A chromatogram is the detector signal that is plotted as function of time and an obtained series of peaks. A typical chromatogram and retention time for a sample

is shown in Figure 2. A retention time is the time after injected sample/analyte to reach the detector that is given the symbol t_R or RT. The small peak on the left with the time of t_M is the unretained compound by the column. In addition, the basic parameters related to the separation are a distribution constant (K), a capacity factor (k′), a selectivity factor (α), a column efficiency (act as plate height (H) and number of theoretical plates (N)), and a column resolution (R_S). A column resolution is the value for separation efficiency of two analytes of A and B compounds or two peaks (see in the Figure 2) in column ($Rs = (t_{R,B}) - (t_{R,A})/1/2(W_A + W_B)$ when $t_{R,A}$ and $t_{R,B}$ are a retention time of peak A and B and W_A and W_B are a width of peak A and B. A resolution (R_S) of 0.75 and 1.0 give overlapping separation of A and B peaks (Figure 2) whereas a resolution of 1.5 provides complete separation. Thus, the resolution factor (R_S) should be grater then 1.5 that it is depend on the improvement of relative factors of a column and a component of mobile phase.

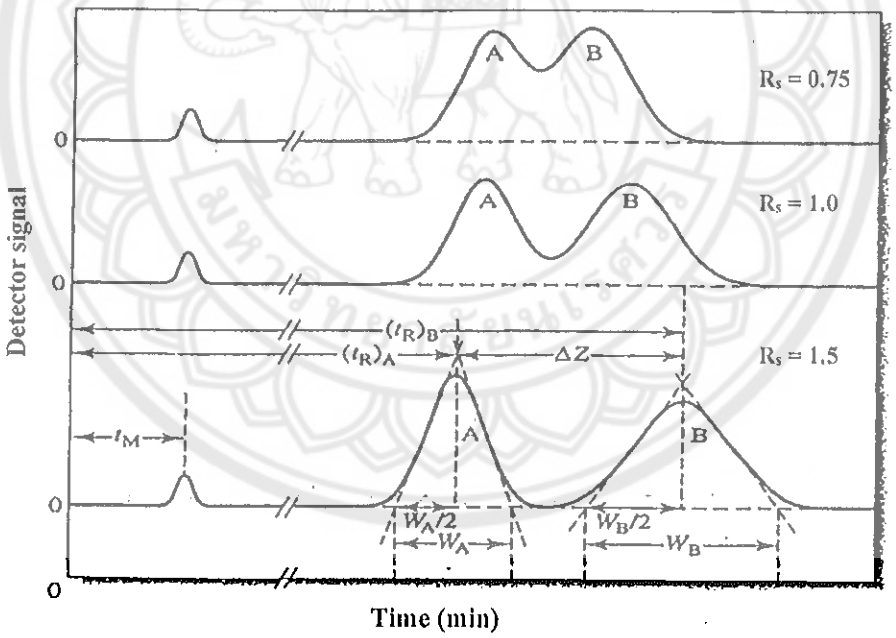


Figure 2 Separation at three resolution values [84]

2. Basic components of HPLC system

The schematic of the five major HPLC components are shown in Figure 3 and their functions are going to be presented [82].

Pumping system: A HPLC **pump** is to propel a liquid (in the mobile phase reservoir) with a constant flow delivery through the **chromatograph** at a specific **flow rate**, expressed in mL min^{-1}. The pump should be made of stainless steel, titanium and resistant minerals and inert to solvents, buffer salts and solutes. Normally, there are two types of the HPLC pump operation: isocratic pump (to deliver constant mobile phase composition) and gradient pump (to deliver variable mobile phase composition).

Injection system: An injector serves to introduce (via a manual at injection valve or an automatic introductions) a liquid sample into a flow stream of a mobile phase without disturbing the column packing. Typical sample volumes are 5-20 μL. The injector must be able to withstand the high pressure of the mobile phase. The injection system includes a precolomn or a guard column. The precolumn, a small removable section of tubing containing the same packing material as the column, can be used ahead of the analytical column to protect the latter from contamination. The precolumn also acts as a buffer to prevent channel of the packing during injection.

Analytical column: A HPLC column is considered the heart of the chromatograph. The success or failure of a particular analysis (mainly qualitative and quantitative) depends on the choice of column or column's stationary phase to separate the sample components using various physical and chemical parameters. Usually, the HPLC analytical columns are constructed with stainless steel (internal diameter (i.d.) 1.0-4.6 mm; lengths 15 –250 mm) and are packed with small diameter porous materials usually in the size of 1.0 – 5.0 μm. These porous materials in the column usually have a chemically bonded phase (e.g. C8, C18 and silica) on their surface which interacts with the sample components to separate them from one another.

Detector: A detector is the critical part of HPLC for detection and identification of sample separated-components and sends its corresponding electrical signal to a computer data station. The detector also records the retention time of components based on the order in which they come out the column. This output can then be analyzed based on peak area to determine the exact nature of the sample's components.

Data system: This system includes a computer, a data processor and a recorder. For modern HPLC systems, the computer and software not only for controlling all the modules of the HPLC instrument (e.g. mobile phase composition, temperature, flow

rate, injection volume and also acquisition and treatment of output) but also for taking the signal and data from the detector.

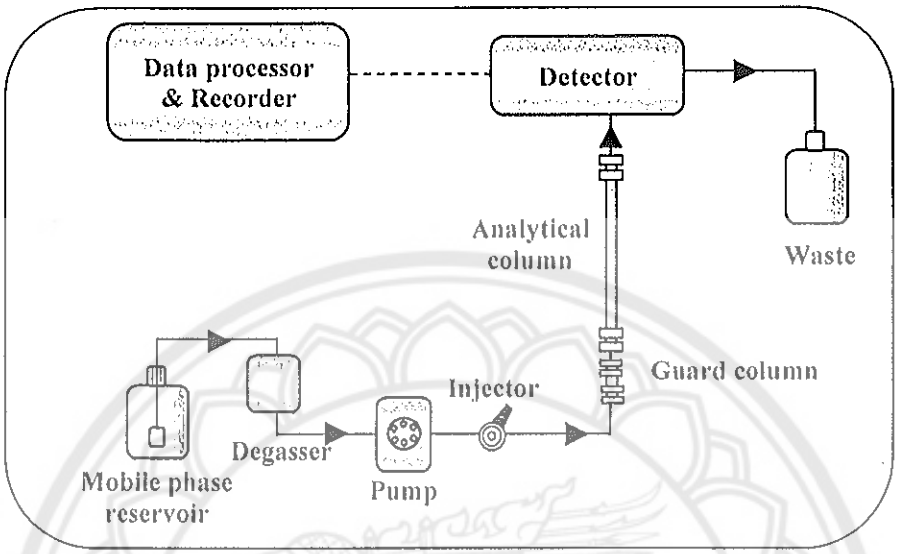


Figure 3 Schematic diagram of a HPLC instrument (adapted from reference [82])

Chromatographic fingerprint analysis

Chromatographic fingerprint analysis [10, 87, 88, 89] is a powerful approach for controlling the quality of herbs or herbal medicines (HMs). Chromatographic fingerprint of HMs is a chromatographic pattern or profile which may feature pharmacological activity or some chemical characteristics of those HMs. The proposed technique is used for identification, authentication, determination and standardization. Chromatographic fingerprint can be perform using techniques such as thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and hyphenated techniques. For HPLC fingerprint analysis, the basic steps of conventional methods for standardization of herbal formulation are including 1) preliminary testing for the presence of chemical groups, 2) quantification of interested chemical groups and 3) establishment of HPLC fingerprint pattern or profile based upon single or multiple peak markers. These peak markers can be classified in two groups of component-based and pattern-based approaches.

Component-based approach aims to make the relative compositions with some known chemical components which include a marker approach and a multi-compound approach. The marker approach takes into account the herbs with known components. This approach is identifying herbal extracts by measuring the concentration of one or few markers or active compounds (biomarkers). The multi-component approach employs the relative compositions of many or all identified components with known compounds, which is the chemical profile of the sample, to represent the sample. It is popularly used for many other complex sample including herbs.

Pattern-based approach considers the whole chromatographic profile as a feature. It includes a pattern approach and a multi-pattern approach. The pattern approach is assessed the whole chemical information from analytical instrument such as chromatographic profile to establish pattern fingerprint of herb or HMs. The obtain data may be from one-, two- or higher dimension of chromatographic instruments. Even the relation between the pattern and the chemical composition of the sample may be unclear, but the pattern is determined by the chemical components present. This approach is on one type of pattern, for example, chemical fingerprints of chromatograms. The multi-pattern approach is combined better characterization of the sample from several analytical methods for quality control.

Continuous flow system

The continuous flow analysis system deals with any automatic method in which concentration of analyte is determined continuously in a stream of fluid (liquid or gas). Over past 30 years, this technique was developed into a wide array of generation as following.

Flow injection analysis or FIA [84, 90] is based on the injection of a liquid solution (sample/reagent) into a moving, non-segmented carrier stream of a suitable liquid. The injected sample zone disperses and reacts with the components of the carrier steam at reaction coil. And then, they are transported toward a detector and continuously record the signal (e.g. absorbance, electrode potential, or other physical parameters). The simplest FIA system (Figure 4 (a)) consists of pump, injection port, reactor and connector and detector and recorder. Pump system propels the carrier stream through a small tube. It consists of a set of rollers and tubing which are used to maintain a constant

flow rate and corresponding constant residence times. Injection port injects sample/reagent solution into the carrier stream which the solution must have reproducible sample volume and the injections must not disturb the flow of the carrier stream. Reactors and connectors are made of plastic tubing, which can be coiled, knitted, or knotted to decrease zone dispersion. The most suitable tube is Teflon because of being chemically resistant and adsorbs the least solutes on surface. Detector and recorder detect and record the output of sample zone. The detector has been applied in several techniques such as atomic absorption and emission instruments, fluorometers, electrochemical system, refractometers, spectrophotometers and photometers. A typical recorder output has the form (Figure 4 (b)) of a peak, the height (H), width (W), or area (A) of which is related to the concentration of the analyte.

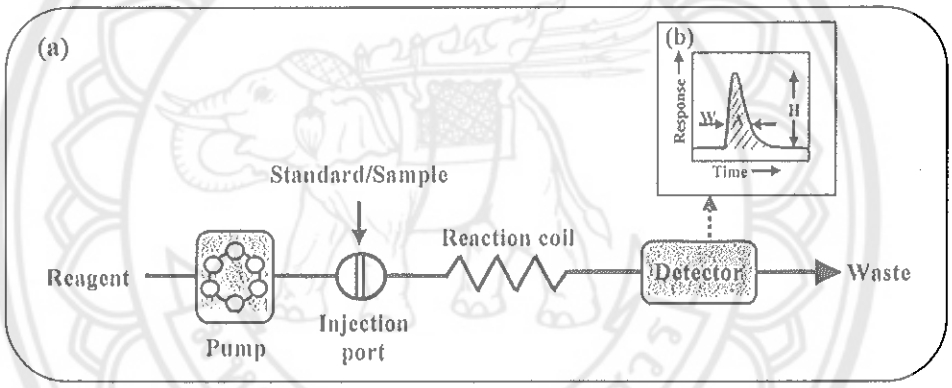


Figure 4 A basic diagram of (a) flow injection analysis (FIA) system and (b) the output has the form of a peak, H – peak height, W – peak width, and A – peak area (adapted from reference [90])

Furthermore, this technique is based on a combination of three principles of sample injection, controlled dispersion of the injected sample zone and reproducible timing. **Sample injection** is the introduced sample by a reproducible volume at precisely timed intervals into a stream. **Controlled dispersion** is precise control of flow dispersion of sample zone when it moves toward detector. The flow dispersion is expressed in terms of dispersion coefficient (D) which is ratio of analyte concentrations of the injected sample (C^0) and detector (C) ($D = C^0/C$). It is divided into limited, medium and large dispersions. Limited dispersion (D = 1-3) has found in flow injection

techniques for high-speed feeding of such detector systems as flame atomic absorption and emission, inductively coupled plasma as well as voltametry. The sample solutions are aspirated directly in to the instrument and signal is measured. Medium dispersion (D = 3-10) is used for spectrophotometric or fluorometric detection. And large dispersion (D > 10) is employed for extensive dilution of sample and reagent and titration technique. Moreover, the other factors can be influenced on dispersion such as sample volume, tube length and flow rate. **Reproducible timing** is precise movement from the injection point toward and into the detector.

Sequential injection analysis or SIA [90] is based on discontinuous flow, which is programmed to move forward as well as backward. The good points of this flow form are to promote mixing of the sequentially injected zones and to allow reaction rate measurements to be carried out in stop flow mode. The basic equipment of SIA (Figure 5) consists of a syringe pump, a holding coil, a multi-position selection valve and a detector. Similar to FIA, the SIA readout is resulted of fluidically controlled dispersion and ensuring chemical reactions. The basic procedure of SIA is microliter volumes of sample and reagent solutions are sequentially stacked within the holding coil and by following flow reversal transported into the detector.

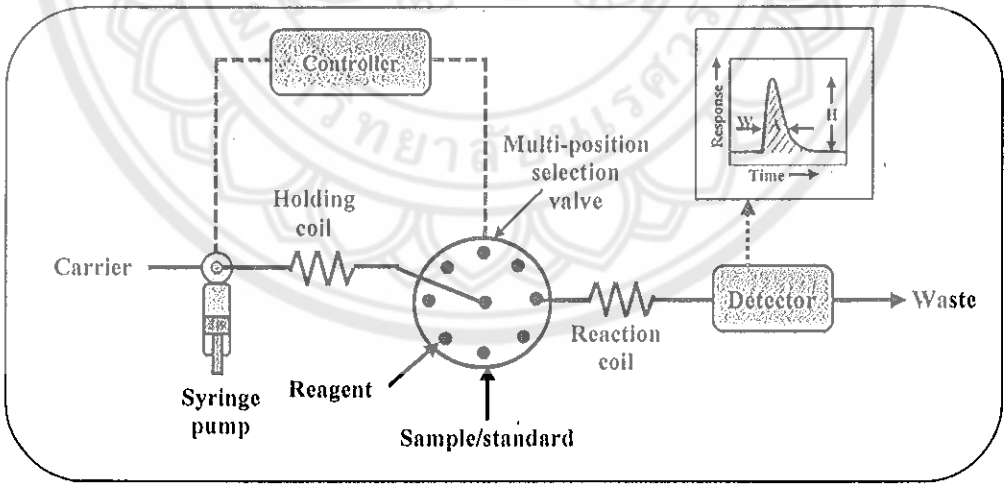


Figure 5 A diagram of the basic sequential injection analysis (SIA) system (adapted from reference [90])

The flow injection (FIA) and sequential injection (SIA) are similar in precise volume of loading solution, operational in automatic made and low chemical

consumption. However, limited FIA method is used high amounts of reagent or chemical even though it is simple and low cost devices. In addition, SIA are expensive and high quality of pump, selection valve and computer controller. To compromise the limitation of both methods, the semi-automatic, simple and cost-effective instruments of the continuous flow systems or hydrodynamic sequential injection or multicommutated flow system were assembled and applied to determine antioxidant capacity and total phenolic compounds using ABTS and FC assays.

CHAPTER III

RESEARCH METHODOLOGY

Instruments

1. High performance liquid chromatography: 1100 series, Agilent, USA
2. UV-Vis spectrophotometer (double-beam): V-650 model, Jasco, Japan
3. Continuous flow injection system designed and constructed by research groups of Chanyud Kritsunankul, Orawan Kritsunankul and Jaroon Jukmunee
4. Analytical balance (4 digit): BS 224S, Satorius, Germany
5. Analytical balance (5 digit): XS105 Dualrange, Mettler Toledo, USA
6. Micropipette: 10 – 100 and 100 – 1000 µL Boeco, Thailand

Chemicals

All chemicals were analytical reagent (AR) and HPLC grades. The chemicals are listed as follows:

1. Acetic acid [$C_2H_4O_2$]: 100 % (glacial), AR grade, Merck, Germany
2. Acetonitrile [C_2H_3N]: 99.9 %, HPLC grade, Fisher Scientific, USA
3. 2,2′-Azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt or ABTS [$C_{18}H_{24}N_6O_6S_4$]: ≥ 98 %, HPLC grade, Sigma-Aldrich, USA
4. (+)-Catechin [$C_{15}H_{14}O_6$]: 99 %, HPLC grade, Sigma-Aldrich, USA
5. (-)-Epicatechin [$C_{15}H_{14}O_6$]: 95 %, HPLC grade, Sigma-Aldrich, USA
6. Epigallocatechin gallate [$C_{22}H_{18}O_{11}$]: 95 %, HPLC grade, Sigma- Aldrich, USA
7. Folin-Ciocalteu's phenol reagent (containing: water, lithium sulphate, sodium tungstate, phosphoric acid, hydrochloric acid and brom): Merck, Germany
8. Formic acid [CH_2O_2]: 98-100 %, AR grade, Merck, Germany
9. Gallic acid [$C_7H_6O_5.H_2O$]: ≥98 %, HPLC grade, Sigma-Aldrich, USA
10. (-)-Gallocatechin [$C_{15}H_{14}O_7$]: 97 %, HPLC grade, Sigma-Aldrich, USA
11. Potassium peroxodisulfate [$K_2S_2O_8$]: < 99 %, AR grade, Merck, Germany
12. Methanol [CH_3OH]: 99.9%, HPLC grade, BDH, England

13. Potassium dihydrogen orthophosphate [KH_2PO_4]: 99.9 %, AR grade, Fisher Scientific, USA

14. Sodium acetate trihydrate [$C_2H_3O_2Na.3H_2O$]: 99.5-100.5 %, AR grade, Merck, Germany

15. Sodium hydroxide [NaOH]: > 97 %, AR grade, Carlo Erba, Italy

Preparation of solutions

All solutions were prepared in ultrapure water with resistivity 18.2 MΩ.cm (Elgastat maxima, England) throughout this work. All stock standard solutions were stored in amber glass bottle and kept at 4 °C.

1. **Stock standard solutions of gallic acid (GA), gallocatechin (GC), catechin (C), epicatechin (EC) and epigallocatechin gallate (EGCG) (1000 mg L^{-1} of each solution)**

A 0.00513 g of GA was dissolved and made up with water into a 5 mL of volumetric flask, while a 0.00515, 0.00507, 0.00556 and 0.00526 g of GC, C, EC and EGCG were dissolved and made up with 99.9% v/v methanol into a 5 mL volumetric flask. A mixed standard solution of GA, GC, C, EC and EGCG was freshly prepared by suitable dilution of each stock standard solution.

2. **HPLC mobile phase solution (acetonitrile:0.1% v/v formic acid = 15:85 v/v**

A mobile phase solution was mixed between a 150 mL of acetonitrile and a 850 mL of 0.1% v/v formic acid into a 1000 mL volumetric flask. Then, the solution was filtrated through a 0.45 μm Nylon membrane filter (Vertical Chromatography, Thailand) and degassed using an ultrasonic bath (Elma, Germany) for 15 min.

3. **ABTS (14 mmol L^{-1}) and potassium peroxodisulfate (10 mmol L^{-1}) solutions**

A 0.0360 g of ABTS was dissolved and made up with water into a 5 mL volumetric flask. And a 0.0135 g of $K_2S_2O_8$ was dissolved and made up with water into a 5 mL volumetric flask.

4. **$ABTS^{\bullet+}$ solution (7 mmol L^{-1} ABTS + 2.45 mmol L^{-1} $K_2S_2O_8$)**

To generate the bluish-green color of the ABTS radical cation ($ABTS^{\bullet+}$) stock solution, a 0.50 mL of 14 mmol L^{-1} ABTS, a 0.245 mL of 10 mmol L^{-1} $K_2S_2O_8$ and a 0.255 mL of water were mixed into a micro-centrifuge tube, kept in the dark for 16 h at room temperature (25±3 °C) and then stored at 4 °C for 7 days. The $ABTS^{\bullet+}$ working

solution was daily prepared by diluting with water to obtain an absorbance value of 0.85±0.02 at 730 nm. The color of $ABTS^{\bullet+}$ solutions in different media solutions is shown in appendix A.

5. Folin-Ciocalteu solution (FC) (FC reagent:water = 1:15 v/v

A 6.7 mL of Folin-ciocalteu's phenol reagent was pipetted and diluted with water into a 100 mL volumetric flask. The color of FC solutions is shown in appendix A.

6. Acetate buffer solution (0.02 mol L^{-1}, pH 4.5)

A 0.73 mL of glacial acetic acid and a 0.98 g of sodium acetate trihydrate were dissolved with water into a 1000 mL volumetric flask.

7. Sodium hydroxide solution (0.25 mol L^{-1})

A 1.00 g of sodium hydroxide was dissolved and made up with water into a 100 mL volumetric flask.

Preparation of sample solutions

1. Banana crude extract of banana wastes

All sample of 54 dried crude extracts of banana wastes (summarized in Table 6 and Appendix B) were extracted using 95% v/v ethanol which were carried out by Kornkanok Ingkaninan (Faculty of Pharmaceutical Sciences, Naresuan University and research groups). These banana wastes were peels, hand stalks and bunch stalks and were collected from Phitsanulok, Sukhothai and Kampheang phet provinces. Each province was collected in the different seasons of summer, winter and rainy reasons.

For HPLC analysis, an approximately 0.04 g of each dried-crude extract was weighed into a micro-centrifuge tube, dissolved with 1.5 mL of 90% v/v methanol, mixed well with ultrasonic bath (S 70H Elmasonic, Elma, Germany) for 10 min at room temperature (27±3 °C), and centrifuged for 5 min at 5000 rpm. After that, its supernatant solution was dried under N_2 gas and then dissolved with 200 µL of 90% v/v methanol into a micro-centrifuge tube. Next, a portion of 50 µL of this extract solution was diluted and made up with 90% v/v methanol into a 200 µL micro-centrifuge tube. Finally, the diluted sample solution was filtrated through a 0.45 µm nylon filter (GAT, Thailand) before it was introduced into the HPLC system.

Table 6 Names and details of 54 samples of dried crude banana extracts used in this work

Crude extracts	Abbreviations	Details
Raw peels	P1 – P9	Collected from Phitsanulok, Sukhothai and Kamphaengphet provinces in the different summer, winter and rainy reasons (as shown in Appendix B)
Ripe peels	PR10 – PR18	
Raw hand stalks	H1 – H9	
Ripe hand stalks	HR10 – HR18	
Raw bunch stalks	B1– B9	
Ripe hand stalks	BR10 -BR18	

2. **Tea and herbal tea**

All 20 dried samples of teas and herbal teas used in this work (see in appendix C) were purchased at local supermarkets in Phitsanulok province. Each sample was ground and separated particles using a sieve device (mesh size in a 20/1 of holes per inch). Then, an approximately 0.50 g of each powder sample was extracted with a 30 mL of hot water (95 °C) for 5 min, filtered through a filter paper (Whatman No.1), cooled to room temperature, and then made up with water into 50 mL volumetric flask. Each extracted-sample solution was kept in dark at 4 °C and analyzed within 12 hours by the continuous flow system used in this work.

Determination of some antioxidant compounds in banana waste extracts by HPLC and chromatogaphic fingerprint analysis

1. **The HPLC system**

The HPLC system (Agilent 1100 series) consisted of a degasser (model G1322A), a quatupump (model G1311A), an autosample series (model 1329°, Agilent 1200 series), a C18 guard column (Vertical chromatography, Thailand: 5 μm, 4.6 mm i.d. x 10 mm length), a C18 analytical column (VertiSepTM pHendure C18: 5 μm, 4.6 mm i.d. x 250 mm length), a photodiode array detector (DAD; model G1315B) and an agilent software.

2. **Optimized conditions of the HPLC system**

The important parameters affected HPLC system for the determination GA, GC, C, EC and EGCG in banana waste extracts were optimized to obtain a good

resolution, short analysis time, high sensitivity, and good precision and accuracy. These parameters were 1) chromatographic separation study of some antioxidant compounds 2) detection wavelengths (varied in the range of 210 - 290 nm) and 3) ratios of mobile phase (varied in ratios of 10:90, 15:85, and 20:80 v/v of acetonitrile and 0.1% v/v formic acid). Finally, the selected conditions were summarized and applied to samples of banana crude extracts. For method validation of the proposed system, a mixed standard solution was added into all samples and reported as percentage recoveries.

3. **The chromatographic fingerprint analysis**

The process of quality control was performed to achieve the chromatographic fingerprint pattern of all 54 samples of banana waste extracts by analysis the HPLC data such as chromatograms and retention times of all samples. This process included 1) the overlapped chromatograms, 2) the normalization of retention times and 3) the selection of representative peak markers by calculation a relative retention time (RRT) to determine similarity of peak marker. This RRT [91, 92] was calculated by RT_{ref}/RT_{detect} when the RT_{ref} and RT_{detect} are a retention time of the reference peak and the detected peak. To confirm the precision of RRT values of each peak marker, the percentage relative standard deviation (%RSD) was calculated and finally reported.

Determination of antioxidant capacity using ABTS assay and total phenolic compounds using FC assay in teas and herbal teas by continuous flow injection systems

1. **Related reactions**

For ABTS assay [93, 94], the determination of antioxidant capacity is based on the scavenging ability of antioxidants to the long-life ABTS radical cation ($ABTS^{\bullet+}$). In this work, the ABTS assay is a GEAC (gallic acid equivalent antioxidant capacity) assay using GA as standard solution (resulted in GA equivalents). In this assay, ABTS is oxidized by $K_2S_2O_8$ to product $ABTS^{\bullet+}$ (a blue-green color solution). And then an antioxidant capacity is measured as the ability of test compound in sample to decrease the color reacting directly with the $ABTS^{\bullet+}$ radical (Figure 6) at 730 nm. Results are expressed relative to GA.

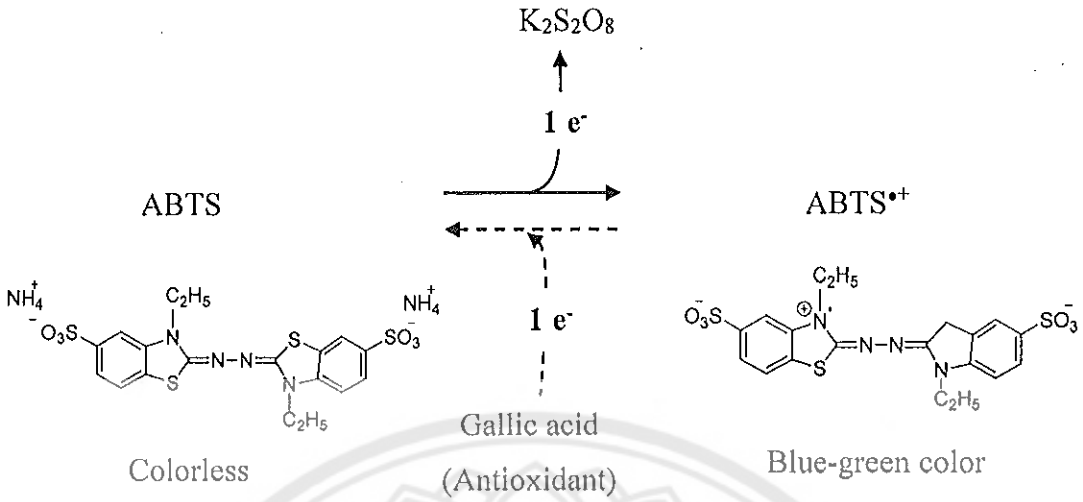


Figure 6 ABTS chemical reaction (adapted from reference [95])

For FC assay [96, 97], it has been generally used as a measure of only phenols (total phenolics) or phenols plus reducing agents plus possibly metal chelators. In this work, the FC assay is based on the oxidation of total phenolic compounds by a molybdotungstophosphoric heteropolyanion reagent (FC reagent) at pH ~ 10 (NaOH usage) yields a blue color product (Mo(VI) to Mo (V)) that exhibit a board light absorption with a maximum at 728 nm (see equation 5 and 6). This absorption wavelength is propartional to the concentation of total phenolic compound in samples and results are expressed in GA equivalents.

$$Na_2WO_4 + Na_2MO_4 \xrightarrow[HCl,\ H_3PO_4]{Li_2SO_4} \underset{\text{FC reagent (yellow)}}{3H_2O{-}P_2O_5{-}13WO_3{-}5MoO_3{-}10H_2O \text{ and } 3H_2O{-}P_2O_5{-}14WO_3{-}4MoO_3{-}10H_2O} \quad (5)$$

$$\text{Mo(VI) (yellow)} + AH \xrightarrow{NaOH} \text{Mo(V) (blue)} \quad (6)$$

2. Study of absorption spectra

Based on ABTS and FC assays, all solutions were measured to obtain maximum wavelength and no absorbed of solvent and other species for the determination of antioxidant capacity and total phenolic compounds by a UV/Vis spectrophotometer (V-650 spectrophotometer, Jasco, Japan) with SpectraManager

software for the analysis. The conditions used were 200 nm, 900 nm, 200 nm min^{-1} and 2 nm of start wavelength, end wavelength, scan speed and scan smooth, respectively.

For the ABTS assay, a ABTS solution (7 mmol L^{-1}), a $K_2S_2O_8$ solution (2.45 mmol L^{-1}), a aqueous $ABTS^{\bullet+}$ solution (7 mmol L^{-1} ABTS + 2.45 mmol L^{-1} $K_2S_2O_8$) (ratio 1:85 v/v), and a mixed solution of $ABTS^{\bullet+}$ solution and GA (0.01 mmol L^{-1}) prepared in water within 7 days and for the FC assay, an aqueous FC solution (ratio 1:10, v:v), a NaOH solution (0.25 mol L^{-1}), a mixed solution of FC + GA (1 mmol L^{-1}), and a mixed solution of FC + GA (1 mmol L^{-1}) + NaOH (0.25 mol L^{-1}) were studied by recording the absorption spectra.

3. **Instrumental setup of the continuous flow systems using ABTS and FC assays**

The continuous flow systems of ABTS and FC assays were designed and constructed by research groups of Chanyud Kritsunankul (Department of Natural Resources and Environment, Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand), Orawan Kritsunankul (Department of Chemistry, Faculty of Science, Naresuan University, Phitsanulok, Thailand) and Jaroon Jakmunee (Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand). These two systems are shown in Figure 7(a) for ABTS assay and Figure 7(b) for FC assay. The systems consisted of a peristaltic pump (P; Masterflex, Coleparmer, USA), two-way solenoid valves (SV_1, SV_2, SV_3 SV_5; Cole-parmer, USA), a three-way solenoid valve (SV_4; Cole-parmer, USA), a flow through cell (FTC; 10 mm path length, Perkin elmer, USA), a UV-Vis spectrophotometer (D; Spectro SC, Labomed, USA), a homemade controller (made by Dr. Chanyud Kritsunankul) and a homemade interface (made by Dr. Jaroon Jakmunee). The SV_1, SV_2, SV_3, SV_4, SV_5 and P were controlled by a homemade controller. A personal computer with in-house built software (Recorder, version 5) and eDAQ chart software were used for collecting data and interpreting peak height, respectively. All tubing for assembling systems was teflon tube of 0.89 mm i.d., except pump tubes.

4. **Procedure of the system using ABTS and FC assays**

The procedure of the system using ABTS assay in Figure 7(a) can be described as follows. The operation of this system was consisted of filling, loading, injection and cleaning steps. Firstly, the carrier (Cs), standard/sample (S) and reagent

(R_A) solutions were filled pass through a RL (or Reaction loop), FTC, waste coil (WC) and W, respectively. After that, the operation cycle of loading and injection steps was started. For the first injection, S and R_A solutions were rapid sequential aspirated through RL and WC, respectively. The S+R_A zone in RL was stopped with an appropriate time and then was pushed to FTC and W, respectively. Next, the second injection was then started to load and inject according to the operating cycle as above. Finally, the system was cleaned with water passing through all solenoid valves to WC and W, respectively. For FC assay, the procedure of the system using FC assay in Figure 7(b) was similar to ABTS assay excepted in loading and injection step. The S, R_F (FC reagent solution) and R_N (NaOH solution) were sequential aspirated into RL and then S+R_F+R_N zone in RL was immediately pushed to FTC and W, respectively.

5. **Optimized conditions of the continuous flow system using ABTS and FC assays**

The several parameters of the systems using ABTS and FC assays were optimized to obtain a wide linear range, good sensitivity, and good accuracy and precision. **For ABTS assay**, these parameters were 1) types of carrier solution (using water and acetate buffer solution (0.02 mol L^{-1}, pH 4.5)), 2) $ABTS^{\bullet+}$ concentrations (varied in the range of 0.6 – 0.9 absorbance value requirement), 3) flow rates of the system (varied in the range of 1.0 – 2.0 mL min^{-1}), 4) stopped time at reaction loop (varied in the range of 0 – 60 s) and 5) reaction loop lengths (varied in the range of 20 - 80 cm). **For FC assay**, there are 1) FC concentrations (varied ratio of FC:water of 1:25, 1:20, 1:15, 1:10, and 1:5, v:v), 2) NaOH concentrations (varied in the range of 0.15 – 0.35 mol L^{-1}), 3) flow rates of the system (varied between 1.6 and 2.0 mL min^{-1}), 4) reaction loop lengths (varied in the range of 20 – 50 cm) and aspiration times in ratios of standard/sample (S), FC solution (R_F) and NaOH solutions (R_N) of 1:1:1, 2:1:1, 3:1:1 and 4:1:1, s:s:s) and 5) stopped times at reaction loop (varied in the range of 0 – 300 s).

Finally, the interference effect was studied for both systems and the selected conditions of the both proposed system were summarized and applied to real samples for the determination of antioxidant capacity and total phenolic compounds using ABTS and FC assays in tea and herbal tea samples. Each sample solution was analyzed in triplicate. Results obtained of the proposed systems were compared with a microplate

reader spectrophotometer (Synergy™ H1 moldel, BioTek® instruments) and evaluated by t-test (at 95 % confidence interval).

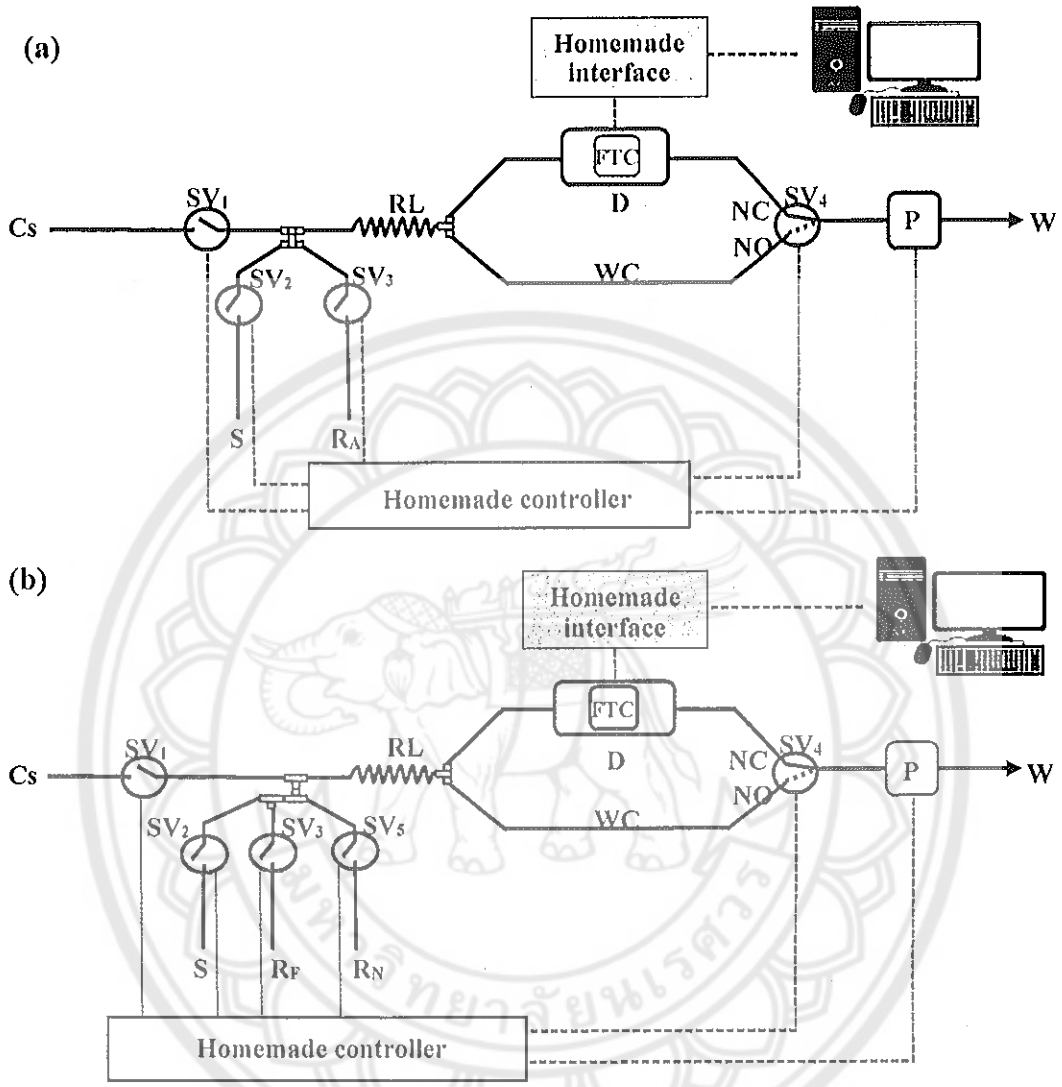


Figure 7 Design of the continuous flow system for the determination of: (a) anti-oxidant capacity using ABTS assay and (b) total phenolic compounds using FC assay (SV – solenoid valve, P – peristaltic pump, Cs – carrier, S – sample/ standard solution, R_A, R_F, R_N – $ABTS^{\bullet+}$, FC, and NaOH reagent solutions, RL – reaction loop, FTC – Flow through cell, D – UV/Vis spectrophotometer, WC – waste coil, and W - waste)

CHAPTER IV

RESULTS AND DISCUSSION

Determination of some antioxidant compounds in banana waste extracts by HPLC and chromatographic fingerprint analysis

1. Optimized conditions of the HPLC system

The preliminay conditions for the determination some antioxidant compounds of gallic acid (GA), gallocatechin (GC), catechin (C), epicatechin (EC) and epigallocatechin gallate (EGCG) by HPLC system are shown in Table 7. These conditions were adapted from the VertiSep^{TM} Phendure C18: Application Note # 686 (Vertical Chromatograpy, Thailand).

Table 7 Preliminary conditions for the determination of GA, GC, C, EC and EGCG by HPLC

Parameters	Conditions used
Analytical column	VertiSepTM pHendure C18 (particle size 5 µm, 4.6 mm i.d. x 250 mm length)
Mobile phase ratio	15:85 v/v of acetonitrile : 0.1% v/v formic acid
Flow rate	1.0 mL min^{-1}
Injection volume	20 µL
Detection wavelength	280 nm
Solvent for standard/sample preparation	90% v/v methanol

1.1 Chromatographic separation study of some antioxidant compounds

The chromatographic separation of all solutions of GA, GC, C, EC and EGCG in 90% v/v methanol were studied. The objective of this study was to screen the characteristic of chromatograms of all chemicals used in this work and to obtain the retention time of all compounds. This study was performed by HPLC system using the preliminary conditions (Table 7). All chromatograms are resulted in Figure 8. It was found that no appearance of interference or no background signal was noticed for all compound solutions. The order of elution was GA, GC, C, EC and EGCG with retention

times (RT) of 3.79 ± 0.04, 4.67 ± 0.12, 8.21 ± 0.36, 11.75 ± 0.49 and 13.32 ± 0.32 min, respectively. Furthermore, these analytes were completely separated from each other within resolution factors (R) more than 1.5. However, under preliminary conditions used, a long analysis time (20 min) per chromatogram and low sensitivity of catechin groups were obtained. Therefore, to decrease an analysis time with acceptable separation and to increase sensitivity, the detection wavelength and the mobile phase ratio were investigated in further study.

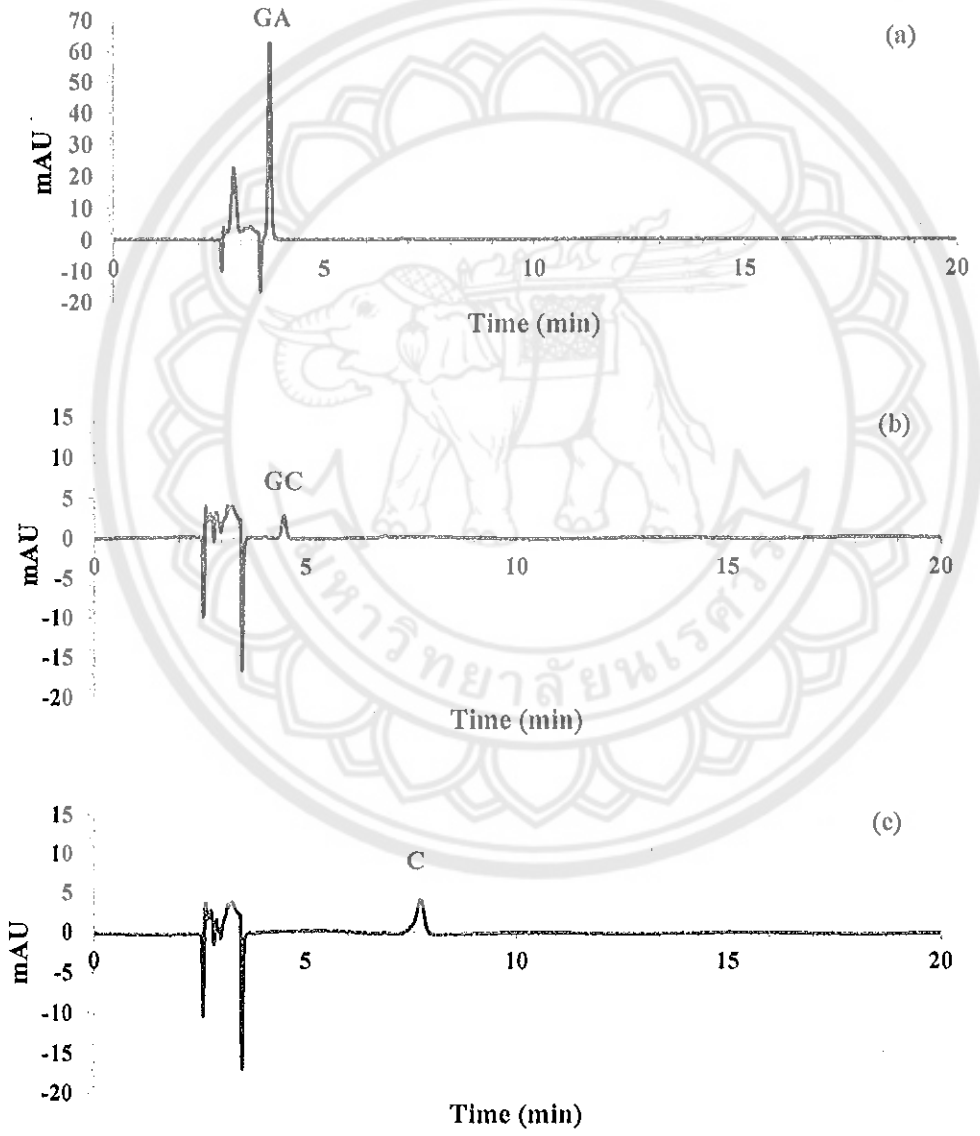


Figure 8 Chromatograms of standard solutions (10 mg L^{-1} of each individual standard solution in 90% v/v methanol)

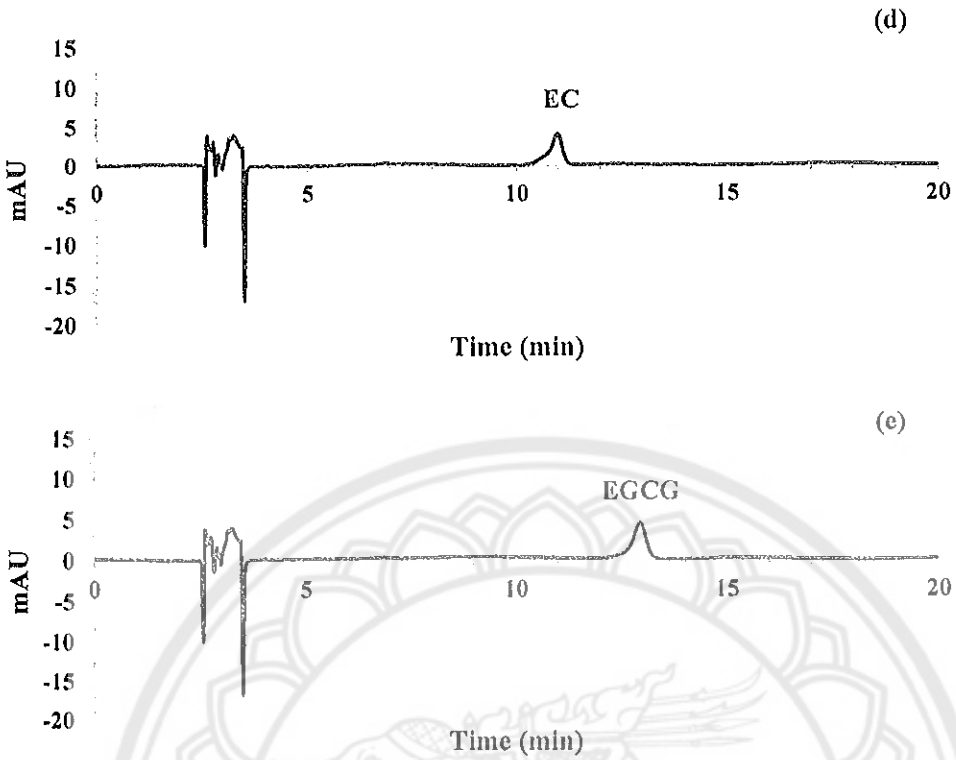


Figure 8 (cont.)

1.2 **Detection wavelength study**

The detection wavelength is one of factors to affect the absorption or sensitivity of all compounds (GA, GC, C, EC and EGCG) and the background of methanol and mobile phase solutions. Thus, the detection wavelengths of all compounds were studied. A mixed standard solution (10 mg L^{-1} of GA, GC, C, EC and EGCG, repectively) in 90% v/v methanol, a 90% v/v methanol and a mobile phase (acetonitrile : 0.1% v/v formic acid; 15:85 v/v) were injected into HPLC system and detected at 210 - 400 nm. Results are shown in Table 8 and Figure 9. Results were found that all compounds absorbed (expressing as peak area) in the range of 210 - 295 nm. The 90% v/v methanol and the moblie phase were resulted the absorption spectra in the range of 210 – 260 nm and 210 – 240 nm, respectively. To obtain high absorption sensitivity and no background of methanol and mobile phase, the detection wavelenth at 275 nm was selected for further study.

Table 8 Peak areas of GA, GC, C, EC and EGCG (10 mg L^{-1} of each compound) in 90% v/v methanol at the different detection wavelengths

Wavelength (nm)	Peak area (n=1)				
	GA	GC	C	EC	EGCG
210	761	862	895	1412	714
215	842	689	625	1012	554
220	767	472	427	675	427
225	571	335	322	504	299
230	360	253	260	404	225
235	172	198	198	273	132
240	104	128	111	160	67
245	113	73	50	52	40
250	147	36	22	35	45
255	193	21	16	26	31
260	251	19	20	33	59
265	293	21	30	46	73
270	322	21	41	66	81
275	336	20	55	91	88
280	312	16	56	93	85
285	271	9	47	77	79
290	230	0	29	48	63
295	185	0	16	23	49

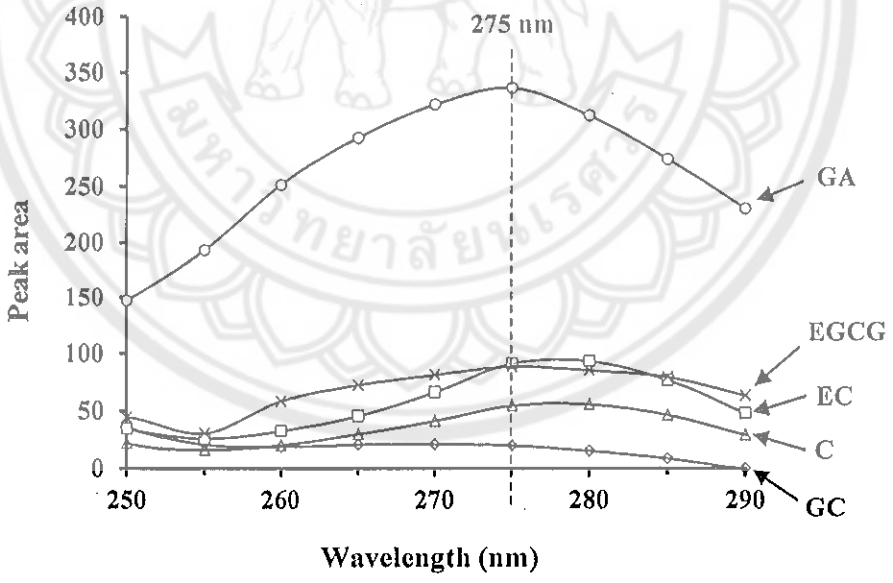


Figure 9 Detection wavelength study of a mixed standard solution of GA, GC, C, EC and EGCG (10 mg L^{-1} of each compound)

1.3 Effect of mobile phase ratios

Commonly, the chromatographic separation of a reversed-phase HPLC is based on polarity of mobile phase. Compounds whose polarity is similar to that of the mobile phase will be preferentially attracted to it and moved faster (decreased the elution time or decreased analysis time per chromatogram) and also affected resolution factors of compounds. To achieve a short analysis time per chromatogram and good resolution factor ($R > 1.5$) for all compounds, the effect of mobile phase ratios was varied at 10:90, 15:85 and 20:80 v/v of acetonitrile (ACN): 0.1% v/v formic acid. A mixed standard solution of GA (4 mg L^{-1}), GC, C, EC and EGCG (8 mg L^{-1} of each compound) was injected into HPLC system with different ratios of mobile phase. Results are shown in Table 9 and Figure 10. It was found that decreasing polarity of mobile phase (or decreasing acetonitrile in mobile phase ratio) decreases analysis time. At a 10:90 and a 15:85 of acetonitrile and 0.1% v/v formic acid were shown a good resolution factors ($R > 1.5$) of all compounds while at a 20:80 v/v of acetonitrile : 0.1% v/v formic acid, GA and GC gave a resolution factor < 1.5. Thus, to compromise good resolution and short analysis time, the ratio of mobile phase at 15:85 v/v was chosen for all studies.

Table 9 Effect of mobile phase ratios on retention time and analysis time for the determination of GA, GC, C, EC and EGCG (4, 8, 8, 8 and 8 mg L^{-1}, respectively) by the HPLC

ACN: Formic acid (v/v)	Retention time (min)					Analysis time (min)
	GA	GC	C	EC	EGCG	
10:90	4.79±0.01*	7.29±0.01	16.85±0.01	31.58±0.01	40.16±0.01	45
15:85	4.21±0.01	5.07±0.01	8.64±0.01	12.16±0.01	14.37±0.01	20
20:80	3.50±0.04	3.65±0.04	5.03±0.05	5.92±0.05	6.56±0.05	10

*$\bar{X}$±SD - mean ± standard deviation (n=3)

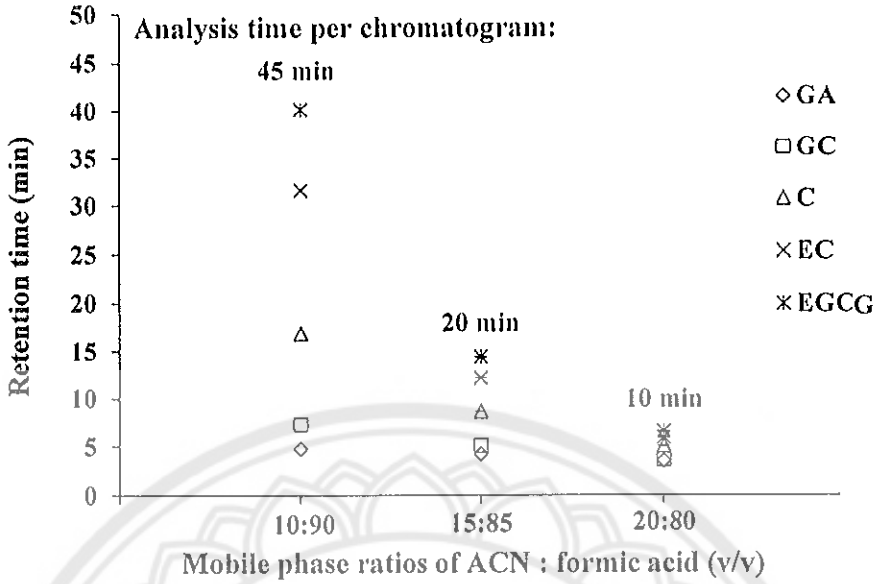


Figure 10 Effect of mobile phase ratios for the determination of GA, GC, C, EC and EGCG (4, 8, 8, 8 and 8 mg L^{-1}, respectively) by the HPLC

1.4 Summary of conditions used of the HPLC system

The optimized conditions of HPLC system for the determination GA, GC, C, EC and EGCG in banana waste extract samples are summarized in Table 10.

Table 10 Conditions used for the determination GA, GC, C, EC and EGCG by the HPLC

Parameters	Conditions used
Analytical column	VertiSepTM pHendure C18 (particle size 5 µm, 4.6 mm i.d. x 250 mm length)
Mobile phase ratio	15:85 v/v of acetonitrile : 0.1% v/v formic acid
Flow rate	1.0 mL min^{-1}
Injection volume	20 µL
Detection wavelength	275 nm
Solvent for standard/sample preparation	90% v/v methanol

1.5 Analytical performance characteristics of the HPLC system for the determination of GA, GC, C, EC and EGCG

Under the optimized conditions used, a mixed standard solutions of GA (0.25 mg L^{-1}) and GC, C, EC and EGCG (0.5 mg L^{-1} of each compound) and other mixed standard solutions were injected (n=3) into HPLC system. The results are obtained in Table 11 – 12 and Figure 11 – 12. It was found that calibration graphs were linear in the ranges of 0.25 – 20 mg L^{-1} for GA and 0.5 – 30 mg L^{-1} for GC, C, EC and EGCG, respectively. Linearity (r^2) was in the range of 0.9991 – 0.9998. The relative standard deviation (RSD) was in the range of 0.1 – 4.1 %. The limit of detection (LOD) and the limit of quantitation (LOQ) were in the ranges of 0.01 – 0.10 and 0.04 – 0.32 mg L^{-1}, respectively. The order of elution was GA, GC, C, EC and EGCG with the retention times of 3.86 ± 0.06, 4.65 ± 0.07, 7.95 ± 0.12, 11.19 ± 0.14 and 13.24 ± 0.17 min, respectively. The analysis time per chromatogram was 20 min.

Table 11 Calibration data for the determination of GA, GC, C, EC and EGCG by the HPLC

GA	Peak area (n=3)					
(mg L^{-1})	1	2	3	$\bar{X}$	SD	%RSD
0.25	9	9	9	9	0.1	0.7
0.5	18	19	19	19	0.2	1.1
1.0	36	36	36	36	0.0	0.0
2.5	95	96	95	95	0.8	0.9
5	204	201	202	203	1.3	0.6
10	403	405	405	404	0.9	0.2
20	833	826	829	829	3.8	0.5
GC	Peak area (n=3)					
(mg L^{-1})	1	2	3	$\bar{X}$	SD	%RSD
0.5	1	1	1	1	0.1	5.4
1.0	2	2	2	2	0.1	2.4
2.5	5	5	5	5	0.0	0.0
5	11	11	11	11	0.2	2.1
10	25	25	25	25	0.2	0.6
20	51	52	51	51	0.5	1.0
30	79	79	80	79	0.2	0.3
C	Peak area (n=3)					
(mg L^{-1})	1	2	3	$\bar{X}$	SD	%RSD
0.5	4	4	4	4	0.1	1.6
1.0	7	7	7	7	0.1	1.4
2.5	22	22	23	22	0.4	1.6
5	45	46	46	45	0.5	1.0
10	89	89	89	89	0.0	0.0

Table 11 (cont.)

C	Peak area (n=3)					
(mg L^{-1})	1	2	3	$\bar{X}$	SD	%RSD
20	169	169	169	169	0.3	0.2
30	250	250	250	250	0.0	0.0
EC	**Peak area (n=3)**					
(mg L^{-1})	1	2	3	$\bar{X}$	SD	%RSD
0.5	5	5	5	5	0.2	3.0
1.0	11	12	11	11	0.3	2.7
2.5	28	28	28	28	0.2	0.5
5	56	58	51	55	3.4	6.3
10	115	113	114	114	1.0	0.9
20	239	236	238	238	1.4	0.6
30	358	342	351	350	8.0	2.3
EGCG	**Peak area (n=3)**					
(mg L^{-1})	1	2	3	$\bar{X}$	SD	%RSD
0.5	5	5	5	5	0.2	3.0
1.0	11	12	12	12	0.3	2.6
2.5	29	30	30	30	0.3	1.1
5	70	68	69	69	0.6	0.9
10	132	133	133	133	0.6	0.4
20	279	280	279	279	0.3	0.1
30	407	400	404	404	3.7	0.9

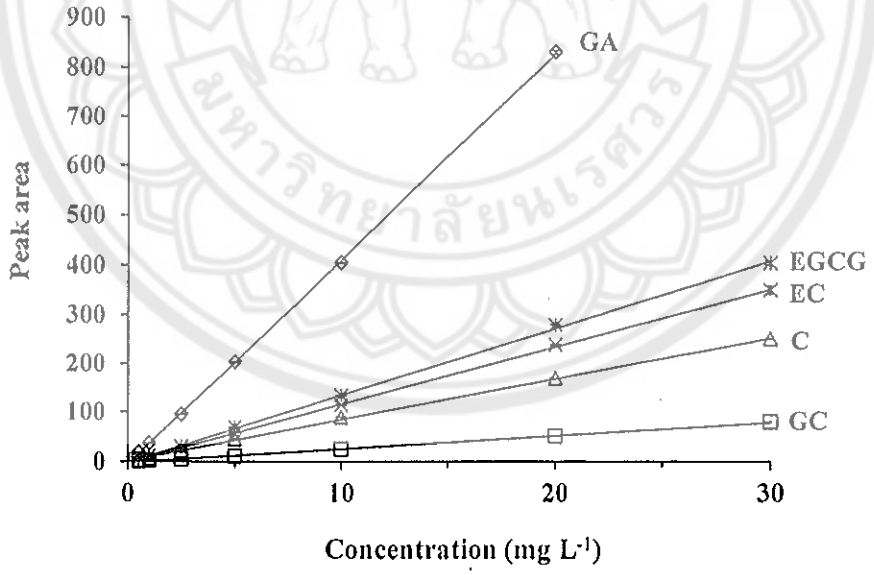


Figure 11 Calibration graphs for the determination of GA, GC, C, EC and EGCG by the HPLC

Table 12 Analytical performance characteristic of the HPLC system for GA, GC, C, EC and EGCG determinations by the HPLC

Antioxidant compounds	Linear range (mg L^{-1})	Linear equation (y=ax+b)	r^2	% RSD[a]	LOD[a] (mg L^{-1})	LOQ[b] (mg L^{-1})
GA	0.25-20	y = 41.57x-5.20	0.9998	0.3-1.5	0.01	0.04
GC	0.5-30	y = 2.65x-1.22	0.9991	0.3-3.0	0.07	0.22
C	0.5-30	y = 8.35x+1.37	0.9993	0.3-2.0	0.10	0.32
EC	0.5-30	y = 11.74x-2.10	0.9991	0.8-4.1	0.01	0.04
EGCG	0.5-30	y = 13.72x-3.53	0.9991	0.1-4.1	0.02	0.07

[a] Limit of detection, calculated from three times standard deviation of the blank signals (SD_b): $3SD_b$/slope (n=11)

[b] Limit of quantitation, calculated from ten times standard deviation of the blank signals (SD_b): $10SD_b$/slope (n=11)

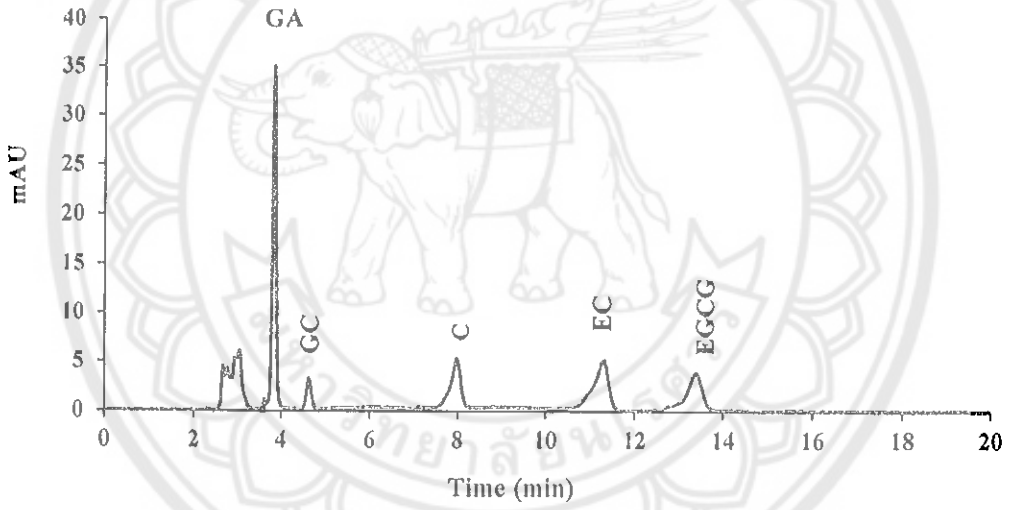


Figure 12 Chromatogram of a mixed standard solution of GA, GC, C, EC and EGCG (5, 10, 10, 10 and 10 mg L^{-1}, respectively) by the HPLC

1.6 Application to real samples of banana crude extracts

The proposed method was applied to determine GA, GC, C, EC and EGCG in raw peel (P), ripe peel (PR), raw hand stalk (H), ripe hand stalk (HR), raw bunch stalk (B) and ripe bunch stalk (BR) of dried crude banana extracts. The accuracy and precision of method were experimented by adding low concentrations (4 mg L^{-1} of GA and 8 mg L^{-1} of GC, C, EC and EGCG, respectively) and high concentrations (8 mg

L^{-1} of GA and 15 mg L^{-1} of GC, C, EC and EGCG, respectively) of standard solutions to all samples of dried crude extracts. The results of concentrations found are summarized in Table 13 - 14 and Figure 13 and typical chromatograms of samples were obtained in Figure 14. It was found that the proposed method can be detected only GA, C, EC and EGCG because GC was interfered from the matrix in the sample. By adding with the mixed standard solution containing low and high concentrations, recoveries (Rec) for GA, C, EC and EGCG were in the range of 70 ± 1 – 115 ± 2, 60 ± 3 – 116 ± 4, 90 ± 5 – 128 ± 1 and 59 ± 1 – 120 ± 5 % with relative standard deviations (RSD) in the ranges of 0.2 – 11.1, 0.2 – 7.7, 0.1 – 8.7 and 0.2 – 5.2 %, respectively. These acceptable recoveries indicate that the purposed method is adequate for the determination of GA, C, EC and EGCG in banana crude extracts. Moreover, it was found that the GA, C and EC were found in all parts of banana crude extracts. The GA and C gave the highest total contents in raw bunch stalk extracts while EC gave the highest total contents in ripe bunch stalk extracts. The EGCG was found in samples of raw peel, ripe peel, raw hand stalk and raw bunch stalk extracts and was the highest total contents in raw hand stalk extracts. The total contents of all compounds were the highest in raw bunch stalk extracts.

Table 13 Contents (mg L^{-1} and mg kg^{-1}) of GA, C, EC and EGCG in banana crude extracts of raw peels (P1 - P9), ripe peels (PR10 – PR 18), raw hand stalks (H1 – H9), ripe hand stalks (HR10 – HR18), raw bunch stalks (B1 – B9) and ripe bunch stalks (BR10 – BR 18), as determined by the HPLC

No.	Sample[a]	Added (mg L^{-1})				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec
1	P1	0	0	0	0	1.8±0.04[b] (18±0.04)[c] [49±1][d]	2.2	-	2.3±0.1 (23±0.1) [64±1]	4.3	-	12.4±0.2 (124±0.2) [345±3]	1.6	-	N.D.[e]	-	-
2		4	8	8	8	5.6±0.04	0.7	95±1	10.2±0.1	1.0	99±2	20.6±0.1	0.5	103±2	8.0±0.2	2.5	100±3
3		8	15	15	15	9.7±0.4	4.1	99±5	16.8±0.2	1.2	97±1	27.4±0.6	2.2	100±4	15.3±0.4	2.6	102±3
4	P2	0	0	0	0	0.3±0.01 (6±0.01) [7±0.2]	3.3	-	1.7±0.1 (34±0.1) [34±1]	5.9	-	9.1±0.3 (183±0.3) [187±6]	3.3	-	2.3±0.1 (47±0.1) [48±0.3]	4.3	-
5		4	8	8	8	4.4±0.1	2.3	103±4	10.1±0.2	2.0	105±2	18.4±0.1	0.5	116±4	10.2±0.4	0.9	99±7
6		8	15	15	15	9.2±0.1	1.1	111±2	18.5±0.3	1.6	112±2	28.3±0.2	0.7	128±1	18.8±0.5	2.7	110±5
7	P3	0	0	0	0	N.D.	-	-	N.D.	-	-	2.8±0.1 (23±0.1) [111±2]	3.6	-	N.D.	-	-
8		4	8	8	8	4.1±0.1	2.4	103±3	8.5±0.2	2.4	106±2	10.3±0.2	1.9	93±2	8.5±0.1	1.2	106±2
9		8	15	15	15	8.1±0.1	1.2	101±2	15.4±0.1	0.6	103±1	18.3±0.1	0.5	103±1	16.0±0.2	1.3	107±1
10	P4	0	0	0	0	0.7±0.02 (3±0.02) [13±0.4]	2.9	-	1.8±0.01 (7±0.01) [35±0.2]	0.6	-	8.0±0.1 (32±0.1) [155±2]	1.3	-	N.D.	-	-
11		4	8	8	8	5.0±0.04	0.8	108±1	10.3±0.1	1.0	106±1	15.9±0.3	1.9	99±3	8.2±0.1	1.3	103±1

Table 13 (cont.)

No.	Sample	Added (mg L^{-1})				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec
12		8	15	15	15	9.3±0.03	0.3	108±1	17.9±0.1	0.6	107±1	22.9±0.3	1.3	99±2	15.3±0.1	0.7	102±1
13	P5	0	0	0	0	10.9±0.1 (44±0.1) [214±2]	0.9	-	N.D.	-	-	9.7±0.2 (39±0.2) [190±4]	2.1	-	N.D.	-	-
14		4	8	8	8	15.0±0.1	0.7	103±2	6.8±0.2	2.9	85±2	17.4±0.1	0.6	96±2	9.6±0.1	1.0	120±1
15		8	15	15	15	18.9±0.1	0.5	100±1	11.5±0.1	0.9	77±1	24.6±0.1	0.4	99±1	15.3±0.2	1.3	102±2
16	P6	0	0	0	0	0.6±0.01 (3±0.01) [12±0.2]	1.7	-	4.7±0.2 (19±0.2) [89±4]	4.3	-	6.1±0.1 (25±0.1) [116±1]	1.6	-	N.D.	-	-
17		4	8	8	8	4.6±0.1	2.2	100±2	13.7±0.2	1.5	112±4	14.0±0.1	0.7	99±1	8.4±0.1	1.2	105±2
18		8	15	15	15	9.5±0.05	0.5	111±1	21.7±0.7	3.2	113±5	21.0±0.6	2.9	99±4	16.0±0.1	0.7	107±1
19	P7	0	0	0	0	0.5±0.02 (2±0.02) [10±0.4]	4.0	-	1.1±0.03 (4±0.1) [21±1]	2.7	-	6.2±0.1 (25±0.1) [119±3]	1.6	-	N.D.	-	-
20		4	8	8	8	4.6±0.01	0.2	103±1	9.3±0.1	1.1	103±2	13.9±0.1	0.7	96±2	8.4±0.1	1.2	105±2
21		8	15	15	15	9.4±0.03	0.3	111±1	17.8±0.2	1.1	111±1	21.3±0.1	0.5	100±1	16.6±0.3	1.8	111±2
22	P8	0	0	0	0	0.7±0.02 (3±0.02) [14±0.3]	2.9	-	1.7±0.02 (7±0.02) [35±0.4]	1.2	-	6.6±0.2 (27±0.2) [136±5]	3.0	-	N.D.	-	-
23		4	8	8	8	4.9±0.1	2.0	105±2	9.7±0.1	1.0	100±2	14.9±0.1	0.7	104±3	9.5±0.2	2.1	119±2
24		8	15	15	15	9.4±0.1	1.1	109±2	16.7±0.1	0.6	100±1	21.7±0.4	1.8	101±2	16.7±0.3	1.8	111±2

Table 13 (cont.)

No.	Sample	Added ($mg\ L^{-1}$)				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec
25	P9	0	0	0	0	3.1±0.1 (12±0.1) [60±3]	3.2	-	10.8±0.2 (43±0.2) [211±3]	1.9	-	9.9±0.2 (40±0.2) [194±5]	2.0	-	N.D.	-	-
26		4	8	8	8	7.0±0.1	1.4	98±3	19.1±0.3	1.6	104±4	17.8±0.2	1.1	99±4	9.3±0.1	1.5	116±2
27		8	15	15	15	11.4±0.1	0.9	104±1	27.2±0.2	0.7	109±3	24.9±0.1	0.4	100±1	16.1±0.3	1.9	107±2
28	PR10	0	0	0	0	N.D.	-	-	N.D.	-	-	2.9±0.1 (23±0.1) [56±1]	3.4	-	N.D.	-	-
29		4	8	8	8	4.1±0.01	0.2	102±1	4.8±0.2	4.2	60±3	11.0±0.1	0.9	101±1	8.0±0.1	1.3	100±2
30		8	15	15	15	8.3±0.2	2.4	104±1	11.2±0.3	2.7	74±2	18.1±0.3	1.7	101±2	15.8±0.2	1.3	105±1
31	PR11	0	0	0	0	2.2±0.1 (11±0.1) [54±2]	4.5	-	N.D.	-	-	7.0±0.1 (35±0.1) [168±1]	1.4	-	1.0±0.03 (5±0.03) [23±0.6]	3.0	-
32		4	8	8	8	6.5±0.1	1.5	107±3	8.5±0.4	4.7	106±5	15.7±0.4	2.5	109±5	8.4±0.3	3.6	92±4
33		8	15	15	15	11.1±0.1	0.9	111±2	17.4±0.5	2.9	116±4	24.2±0.3	1.2	115±3	16.7±0.6	3.6	105±5
34	PR12	0	0	0	0	0.7±0.01 (6±0.01) [27±0.2]	1.4	-	N.D.	-	-	2.3±0.2 (18±0.2) [90±7]	8.7	-	N.D.	-	
35		4	8	8	8	4.7±0.03	0.6	100±1	8.3±0.2	2.4	104±2	10.2±0.1	1.0	99±2	7.9±0.1	1.3	99±1
36		8	15	15	15	9.1±0.01	0.1	105±1	15.4±0.2	1.3	103±1	17.9±0.6	3.4	104±4	14.8±0.1	0.7	99±1

Table 13 (cont.)

No.	Sample	Added ($mg\ L^{-1}$)				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec
37	PR13	0	0	0	0	9.8±0.1 (98±0.1) [477±1]	1.0	-	N.D.	-	-	2.8±0.1 (29±0.1) [139±2]	3.6	-	N.D.	-	-
38		4	8	8	8	14.3±0.1	0.7	112±2	5.1±0.2	3.9	64±3	10.9±0.1	0.9	101±2	8.4±0.1	1.2	105±1
39		8	15	15	15	19.0±0.2	1.0	115±2	10.4±0.1	1.0	70±2	18.3±0.4	2.1	103±3	16.1±0.1	0.6	107±1
40	PR14	0	0	0	0	1.7±0.1 (13±0.1) [64±2]	5.9	-	1.8±0.1 (14±0.1) [69±3]	5.5	-	10.3±0.4 (82±0.4) [399±8]	3.8	-	N.D.	-	-
41		4	8	8	8	5.7±0.05	0.9	100±2	9.7±0.2	2.1	99±4	18.5±0.2	1.1	103±6	9.4±0.2	2.1	118±2
42		8	15	15	15	9.6±0.1	1.0	99±2	17.7±0.2	1.1	106±2	25.9±0.6	2.3	104±4	16.8±0.3	1.8	112±2
43	PR15	0	0	0	0	2.4±0.02 (10±0.02) [48±0.4]	0.8	-	N.D.	-	-	8.3±0.3 (33±0.3) [165±5]	3.6	-	N.D.	-	-
44		4	8	8	8	6.9±0.1	1.4	113±2	6.6±0.1	1.5	83±1	16.6±0.1	0.6	104±3	8.7±0.3	3.4	109±7
45		8	15	15	15	10.9±0.1	0.9	106±2	13.6±0.5	3.7	91±3	24.0±0.6	2.5	104±4	15.4±0.4	2.6	103±5
46	PR16	0	0	0	0	1.5±0.03 (6±0.03) [28±1]	2.0	-	N.D.	-	-	4.7±0.2 (19±0.2) [91±4]	4.3	-	N.D.	-	-
47		4	8	8	8	5.3±0.1	1.9	95±2	8.1±0.3	3.7	101±3	12.6±0.3	2.4	99±5	8.2±0.2	2.4	103±2
48		8	15	15	15	10.2±0.1	1.0	109±2	16.1±0.4	0.6	107±3	20.2±0.2	1.0	103±5	15.9±0.4	2.5	106±3

Table 13 (cont.)

No.	Sample	Added ($mg\ L^{-1}$)				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec
49	PR17	0	0	0	0	1.6±0.02 (7±0.02) [31±0.4]	1.3	-	N.D.	-	-	3.3±0.1 (13±0.1) [63±1]	3.0	-	N.D.	-	-
50		4	8	8	8	6.1±0.1	1.6	113±1	7.3±0.2	2.7	91±2	11.6±0.1	0.8	103±1	8.5±0.2	2.3	106±2
51		8	15	15	15	9.7±0.1	1.0	101±1	15.2±0.3	2.0	101±2	18.3±0.1	0.5	100±1	16.2±0.04	0.2	108±1
52	PR18	0	0	0	0	2.9±0.1 (12±0.1) [59±2]	3.4	-	2.1±0.02 (8±0.02) [42±1]	1.0	-	7.5±0.2 (30±0.2) [152±5]	2.6	-	N.D.	-	-
53		4	8	8	8	6.7±0.1	1.5	95±3	9.9±0.1	1.0	98±2	16.0±0.3	1.9	106±5	8.7±0.2	2.3	109±2
54		8	15	15	15	10.8±0.1	0.9	98±2	17.2±0.2	1.2	101±2	22.7±0.3	1.3	101±3	15.0±0.1	0.7	100±1
55	H1	0	0	0	0	N.D.	-	-	1.8±0.03 (15±0.03) [398±8]	1.7	-	5.1±0.1 (41±0.1) [1124±31]	2.0	-	N.D.	-	-
56		4	8	8	8	4.2±0.1	2.4	105±1	10.1±0.3	3.0	104±4	13.5±0.2	1.5	105±3	8.6±0.3	3.4	108±3
57		8	15	15	15	8.1±0.1	1.2	101±1	16.0±0.1	0.7	95±2	20.1±0.1	0.5	100±2	15.8±0.5	3.2	105±4
58	H2	0	0	0	0	N.D.	3.0	-	6.2±0.5 (139±0.5) [400±5]	8.1	-	11.7±0.3 (292±0.3) [843±3]	2.5	-	N.D.	-	-
59		4	8	8	8	3.3±0.1	3.0	83±3	14.1±0.3	2.1	99±7	19.8±0.2	1.0	101±4	7.8±0.1	1.3	98±2
60		8	15	15	15	6.6±0.3	4.5	83±3	23.0±0.3	1.3	112±2	26.9±0.2	0.7	101±2	13.8±0.3	2.2	92±2

Table 13 (cont.)

No.	Sample	Added (mg L^{-1})				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec
61	H3	0	0	0	0	N.D.	-	-	2.3±0.04 (36±0.04) [188±3]	1.7	-	7.6±0.03 (122±0.03) [634±3]	0.4	-	N.D.	-	-
62		4	8	8	8	4.0±0.2	5.0	100±4	10.1±0.3	2.9	98±4	15.3±0.1	0.7	96±2	8.2±0.1	1.2	103±1
63		8	15	15	15	7.8±0.2	2.6	98±3	17.4±0.4	2.3	101±3	23.1±0.5	2.2	103±4	15.2±0.3	2.0	101±2
64	H4	0	0	0	0	2.0±0.03 (16±0.03) [78±1]	1.5	-	4.0±0.1 (32±0.1) [157±2]	2.5	-	10.2±0.6 (81±0.6) [401±3]	5.8	-	0.6±0.02 (5±0.02) [24±0.4]	3.3	-
65		4	8	8	8	6.0±0.1	1.7	100±2	12.2±0.2	1.6	103±3	18.0±0.04	0.2	98±2	8.4±0.05	0.6	98±1
66		8	15	15	15	10.1±0.1	1.0	101±2	19.4±0.1	0.5	103±1	25.5±0.1	0.4	102±1	15.4±0.2	1.3	99±1
67	H5	0	0	0	0	0.9±0.1 (18±0.1) [91±1]	11.1	-	11.3±0.2 (227±0.2) [1129±4]	1.8	-	11.8±0.2 (236±0.2) [1175±5]	1.7	-	1.6±0.04 (32±0.04) [160±3]	2.5	-
68		4	8	8	8	5.1±0.1	2.0	105±4	19.6±0.3	1.5	104±4	19.6±0.1	0.5	98±3	9.9±0.2	2.0	103±3
69		8	15	15	15	8.3±0.05	0.6	93±2	26.5±0.3	1.1	101±3	26.6±0.2	0.8	99±1	16.6±0.1	0.6	100±1
70	H6	0	0	0	0	0.8±0.02 (13±0.02) [62±2]	2.5	-	11.7±0.1 (186±0.1) [920±1]	0.9	-	12.9±0.2 (206±0.2) [1018±5]	1.5	-	1.2±0.01 (18±0.01) [91±1]	0.8	-
71		4	8	8	8	4.8±0.1	2.1	100±4	19.6±0.4	2.0	99±5	20.8±0.2	1.0	99±4	9.2±0.1	1.1	100±1
72		8	15	15	15	8.4±0.4	3.9	95±6	26.8±0.2	0.7	101±3	27.8±0.3	1.1	99±2	16.2±0.1	0.6	100±1

Table 13 (cont.)

No.	Sample	Added (mg L^{-1})				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec
73	H7	0	0	0	0	0.9±0.05 (9±0.05) [43±1]	5.5	-	6.8±0.2 (69±0.2) [325±5]	2.9	-	11.8±0.3 (118±0.3) [558±2]	2.5	-	N.D.	-	-
74		4	8	8	8	4.0±0.1	2.5	78±3	15.1±0.3	2.0	104±5	20.0±0.2	1.0	103±4	5.7±0.3	5.2	71±4
75		8	15	15	15	7.2±0.2	2.8	79±3	22.7±0.3	1.3	106±3	27.2±0.4	1.5	103±3	9.9±0.3	3.0	66±3
76	H8	0	0	0	0	N.D.	-	-	7.2±0.2 (29±0.2) [141±4]	2.7	-	12.8±0.2 (51±0.2) [250±4]	1.6	-	N.D.	-	-
77		4	8	8	8	3.9±0.02	0.5	98±1	15.7±0.1	0.6	106±3	21.2±0.1	0.5	105±3	8.4±0.1	1.2	105±1
78		8	15	15	15	8.4±0.1	1.2	105±1	23.3±0.3	1.3	107±2	28.7±0.1	0.3	106±1	16.2±0.1	0.6	108±1
79	H9	0	0	0	0	1.6±0.02 (13±0.02) [63±1]	1.3	-	11.8±0.3 (94±0.3) [459±5]	2.5	-	7.3±0.3 (58±0.3) [283±5]	4.1	-	N.D.	-	-
80		4	8	8	8	6.0±0.1	1.7	110±2	20.3±0.3	1.5	106±5	15.5±0.3	1.9	103±5	8.7±0.3	3.4	109±3
81		8	15	15	15	10.0±0.1	1.0	105±2	28.2±0.1	0.4	109±2	21.9±0.1	0.5	97±2	16.8±0.3	1.8	112±3
82	HR10	0	0	0	0	N.D.	-	-	N.D.	-	-	2.3±0.05 (18±0.05) [272±6]	2.2	-	N.D.	-	-
83		4	8	8	8	4.3±0.1	2.3	108±1	8.0±0.1	1.3	100±1	10.4±0.2	1.9	102±3	8.2±0.1	1.2	103±2
84		8	15	15	15	8.2±0.04	0.5	103±1	15.0±0.2	1.3	100±2	16.9±0.2	1.2	97±2	15.7±0.04	0.2	105±1

Table 13 (cont.)

No.	Sample	Added ($mg\ L^{-1}$)				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec
85	HR11	0	0	0	0	N.D.	-	-	3.6±0.1 (86±0.1) [94±1]	2.8	-	11.4±0.2 (273±0.2) [298±4]	1.8	-	N.D.	-	-
86		4	8	8	8	3.5±0.2	5.7	88±4	12.3±0.3	2.4	109±3	19.5±0.2	1.0	101±3	8.7±0.2	2.3	109±2
87		8	15	15	15	8.4±0.1	1.2	105±2	20.7±0.4	1.9	114±3	28.7±0.3	1.0	115±2	15.9±0.4	2.5	106±3
88	HR12	0	0	0	0	1.6±0.03 (18±0.03) [89±3]	1.9	-	6.5±0.2 (74±0.2) [370±5]	3.0	-	13.5±0.1 (155±0.1) [771±3]	0.7	-	N.D.	-	-
89		4	8	8	8	5.2±0.04	0.8	90±3	13.8±0.2	1.4	91±4	21.3±0.1	0.5	97±2	8.2±0.1	1.4	103±1
90		8	15	15	15	8.8±0.1	1.1	90±2	21.2±0.1	0.5	98±1	27.7±0.4	1.4	95±2	14.6±0.5	3.4	97±3
91	HR13	0	0	0	0	6.5±0.2 (26±0.2) [122±3]	3.1	-	N.D.	-	-	8.9±0.1 (36±0.1) [167±1]	1.1	-	N.D.	-	-
92		4	8	8	8	10.4±0.1	1.0	98±5	7.9±0.1	1.3	99±1	16.5±0.1	0.6	95±2	8.4±0.2	2.4	105±3
93		8	15	15	15	14.5±0.1	0.7	100±1	15.4±0.1	0.5	103±1	23.9±0.3	1.3	100±2	15.9±0.1	0.6	106±2
94	HR14	0	0	0	0	0.2±0.01 (8±0.01) [43±1]	5.0	-	0.4±0.02 (15±0.02) [73±4]	5.0	-	11.1±0.3 (445±0.3) [2178±6]	2.7	-	N.D.	-	-
95		4	8	8	8	3.8±0.2	5.3	90±5	9.1±0.3	3.3	109±7	19.6±0.4	2.0	107±7	N.D.	-	-
96		8	15	15	15	7.0±0.1	1.4	85±2	16.4±0.4	2.4	107±6	28.2±0.2	0.7	114±3	N.D.	-	-

Table 13 (cont.)

No.	Sample	Added ($mg\ L^{-1}$)				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec
97	HR15	0	0	0	0	0.5±0.02 (5±0.02) [24±0.3]	4.0	-	2.7±0.1 (27±0.1) [139±2]	3.7	-	9.3±0.5 (93±0.5) [472±3]	5.4	-	N.D.	-	-
98		4	8	8	8	4.9±0.2	4.1	110±5	11.9±0.3	2.5	115±4	19.0±0.3	1.6	121±4	8.3±0.1	1.2	104±1
99		8	15	15	15	8.9±0.1	1.1	105±3	19.0±0.2	1.1	109±2	28.4±0.2	0.7	127±2	14.7±0.2	1.4	98±1
100	HR16	0	0	0	0	1.2±0.04 (5±0.04) [23±1]	3.3	-	4.1±0.2 (16±0.2) [78±3]	4.9	-	13.1±0.2 (53±0.2) [251±5]	1.5	-	N.D.	-	-
101		4	8	8	8	4.6±0.05	1.1	85±2	11.9±0.1	0.8	98±3	21.0±0.2	1.0	99±4	8.0±0.1	1.3	100±1
102		8	15	15	15	8.5±0.1	1.2	91±1	19.7±0.5	2.5	104±3	28.1±0.3	1.1	100±3	15.3±0.2	1.3	102±1
103	HR17	0	0	0	0	1.3±0.02 (5±0.02) [26±1]	1.5	-	N.D.	-	-	11.8±0.2 (47±0.2) [239±3]	1.7	-	N.D.	-	-
104		4	8	8	8	4.6±0.04	0.9	83±1	8.2±0.1	1.2	103±1	19.8±0.2	1.0	100±3	6.9±0.2	2.9	86±2
105		8	15	15	15	8.1±0.1	1.2	85±1	14.9±0.2	1.3	99±1	27.0±0.2	0.7	101±2	14.1±0.6	4.3	94±4
106	HR18	0	0	0	0	0.7±0.03 (2.6±0.03) [13±1]	4.3	-	7.3±0.1 (29±0.1) [149±2]	2.4	-	7.7±0.1 (31±0.1) [156±2]	1.3	-	N.D.	-	-
107		4	8	8	8	4.8±0.04	0.8	103±1	16.3±0.4	2.4	113±5	16.1±0.2	1.2	105±3	9.2±0.2	2.2	115±3
108		8	15	15	15	8.9±0.1	1.1	103±1	24.7±0.3	1.2	116±3	24.2±0.4	1.7	110±5	18.1±0.8	4.4	120±5

Table 13 (cont.)

No.	Sample	Added (mg L^{-1})				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec
109	B1	0	0	0	0	3.9±0.1 (56±0.1) [164±2]	1.2	-	10.3±0.1 (150±0.1) [438±5]	1.0	-	12.8±0.1 (187±0.1) [546±5]	0.8	-	N.D.	-	-
110		4	8	8	8	7.9±0.02	0.3	101±2	18.3±0.1	0.5	100±4	20.8±0.1	0.5	100±4	7.8±0.1	1.3	98±1
111		8	15	15	15	11.8±0.03	0.3	99±1	26.0±0.2	0.7	105±3	27.9±0.1	0.4	101±2	13.8±0.2	1.4	92±1
112	B2	0	0	0	0	5.0±0.02 (119±0.02) [249±1]	0.4	-	7.3±0.3 (175±0.3) [367±13]	4.1	-	9.3±0.1 (223±0.1) [467±2]	1.1	-	N.D.	-	-
113		4	8	8	8	9.3±0.03	0.3	107±7	15.2±0.3	2.0	99±1	17.9±0.2	1.1	108±5	8.7±0.1	1.2	109±7
114		8	15	15	15	13.8±0.04	0.3	110±4	24.4±0.2	0.8	114±5	27.1±0.3	1.1	119±4	14.9±0.3	2.0	99±5
115	B3	0	0	0	0	5.1±0.04 (37±0.04) [145±1]	0.8	-	13.9±0.2 (101±0.2) [394±4]	1.4	-	7.1±0.1 (52±0.1) [202±1]	1.4	-	N.D.	-	-
116		4	8	8	8	9.4±0.1	1.1	108±5	21.6±0.1	0.5	96±4	15.4±0.1	0.6	104±2	8.5±0.2	2.5	106±3
117		8	15	15	15	13.9±0.05	0.4	110±2	28.9±0.3	1.0	100±4	22.4±0.4	1.8	102±5	15.0±0.1	0.9	100±2
118	B4	0	0	0	0	2.0±0.02 (20±0.02) [101±1]	1.0	-	8.2±0.1 (82±0.1) [421±4]	1.2	-	13.4±0.04 (134±0.04) [686±2]	0.3	-	N.D.	-	-
119		4	8	8	8	5.9±0.1	1.7	98±4	16.8±0.2	1.2	108±4	21.4±0.1	0.5	100±4	7.9±0.2	2.5	99±3
120		8	15	15	15	9.9±0.1	1.0	99±4	23.1±0.2	0.9	99±3	28.3±0.2	0.7	99±3	12.8±0.4	3.1	85±3

Table 13 (cont.)

No.	Sample	Added ($mg\ L^{-1}$)				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec
121	B5	0	0	0	0	0.8±0.01 (66±0.01) [337±2]	1.3	-	9.0±0.02 (722±0.02) [3672±5]	0.2	-	1.8±0.04 (144±0.04) [730±13]	2.2	-	0.3±0.01 (27±0.01) [139±1]	3.3	-
122		4	8	8	8	5.2±0.1	1.9	111±5	16.8±0.1	0.6	98±4	9.7±0.3	3.1	99±3	8.7±0.2	2.3	105±4
123		8	15	15	15	9.1±0.1	1.1	104±3	25.3±0.05	0.2	109±3	18.1±0.2	1.1	109±5	16.1±0.1	0.6	105±3
124	B6	0	0	0	0	1.1±0.02 (44±0.02) [219±1]	1.8	-	7.8±0.04 (311±0.04) [1538±4]	0.5	-	4.3±0.03 (172±0.03) [850±5]	0.7	-	0.3±0.01 (10±0.01) [51±2]	3.3	-
125		4	8	8	8	5.0±0.1	2.0	98±3	15.9±0.1	0.6	102±3	12.8±0.1	0.8	106±4	8.5±0.3	3.5	103±3
126		8	15	15	15	9.6±0.1	1.0	106±1	22.8±0.1	0.4	100±1	19.5±0.2	1.0	101±1	15.9±0.3	1.9	104±2
127	B7	0	0	0	0	N.D.	-	-	2.4±0.1 (38±0.1) [184±5]	4.2	-	12.2±0.1 (195±0.1) [940±4]	0.8	-	N.D.	-	-
128		4	8	8	8	4.2±0.2	4.8	105±4	10.6±0.2	1.9	103±4	20.6±0.05	0.2	105±3	N.D.	-	-
129		8	15	15	15	7.9±0.1	1.3	99±2	17.4±0.2	1.1	100±2	27.5±0.1	0.3	102±3	N.D.	-	-
130	B8	0	0	0	0	4.2±0.1 (34±0.1) [172±1]	2.4	-	5.7±0.1 (46±0.1) [233±3]	1.8	-	11.7±0.1 (93±0.1) [475±3]	0.9	-	N.D.	-	-
131		4	8	8	8	8.3±0.1	1.2	103±3	13.7±0.3	2.2	100±4	19.7±0.2	1.0	100±3	N.D.	-	-
132		8	15	15	15	12.6±0.1	0.8	105±1	20.8±0.3	1.4	101±3	26.8±0.3	1.1	101±2	N.D.	-	-

Table 13 (cont.)

No.	Sample	Added ($mg\ L^{-1}$)				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec
133	B9	0	0	0	0	N.D.	-	-	1.7±0.02 (69±0.02) [348±7]	1.2	-	10.1±0.1 (405±0.1) [2036±3]	1.0	-	N.D.	-	-
134		4	8	8	8	3.6±0.1	2.8	89±1	10.1±0.2	2.0	105±6	18.2±0.3	1.6	102±8	N.D.	-	-
135		8	15	15	15	7.0±0.1	1.4	88±1	16.8±0.1	0.6	101±3	25.2±0.2	0.8	101±4	N.D.	-	-
136	BR10	0	0	0	0	1.2±0.02 (10±0.02) [330±6]	1.7	-	N.D.	-	-	3.0±0.1 (24±0.1) [817±28]	3.2	-	N.D.	-	-
137		4	8	8	8	5.1±0.01	0.2	98±1	7.7±0.1	1.3	96±1	11.1±0.1	0.9	101±2	8.9±0.1	1.1	111±1
138		8	15	15	15	9.1±0.04	0.5	99±1	12.1±0.04	0.3	81±1	17.4±0.2	1.1	96±1	15.8±0.2	1.3	105±4
139	BR11	0	0	0	0	1.5±0.02 (179±0.02) [171±2]	1.3	-	1.3±0.1 (158±0.1) [150±5]	7.7	-	13.9±0.2 (1661±0.2) [1582±23]	1.4	-	N.D.	-	-
140		4	8	8	8	5.4±0.1	1.8	98±4	9.7±0.1	1.0	105±2	21.1±0.1	0.5	90±5	5.9±0.1	1.7	73±7
141		8	15	15	15	8.7±0.1	1.1	90±2	17.5±0.8	4.6	108±6	29.3±0.2	0.7	103±2	10.5±0.1	1.0	70±5
142	BR12	0	0	0	0	N.D.	-	-	N.D.	-	-	10.8±0.1 (216±0.1) [1685±10]	0.9	-	N.D.	-	-
143		4	8	8	8	N.D.	-	-	8.2±0.2	2.4	103±3	20.0±0.2	1.0	115±5	N.D.	-	-
144		8	15	15	15	N.D.	-	-	16.4±0.04	0.2	109±2	28.0±0.2	0.7	114±2	N.D.	-	-

Table 13 (cont.)

No.	Sample	Added (mg L^{-1})				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec	mg L^{-1} [mg kg^{-1}]	%RSD	%Rec
145	BR13	0	0	0	0	2.2±0.03 (7±0.03) [139±1]	1.4	-	0.7±0.02 (2±0.02) [42±2]	2.9	-	11.0±0.2 (37±0.2) [684±3]	1.8	-	N.D.	-	-
146		4	8	8	8	6.1±0.1	1.6	98±3	8.6±0.3	3.5	99±4	19.5±0.3	1.5	106±4	6.0±0.2	3.3	75±3
147		8	15	15	15	10.6±0.1	0.9	105±2	15.7±0.2	1.3	100±2	28.0±0.5	1.8	113±4	8.9±0.1	1.1	59±1
148	BR14	0	0	0	0	1.7±0.02 (7.0±0.02) [36±1]	1.2	-	4.4±0.1 (18±0.1) [90±2]	2.3	-	9.3±0.2 (37±0.2) [192±4]	2.2	-	N.D.	-	-
149		4	8	8	8	5.8±0.02	0.3	103±1	12.6±0.2	1.6	103±2	17.5±0.1	0.6	103±3	8.2±0.1	1.2	103±2
150		8	15	15	15	10.2±0.1	1.0	106±1	20.1±0.2	1.0	105±1	24.2±0.2	0.8	99±2	15.9±0.2	1.3	106±1
151	BR15	0	0	0	0	N.D.	-	-	N.D.	-	-	12.1±0.1 (388±0.1) [1881±5]	0.8	-	N.D.	-	-
152		4	8	8	8	4.2±0.03	0.7	105±1	8.9±0.2	2.2	111±2	20.0±0.2	1.0	99±3	N.D.	-	-
153		8	15	15	15	7.9±0.2	2.5	99±3	16.0±0.2	1.3	107±2	26.8±0.2	0.7	98±2	N.D.	-	-
154	BR16	0	0	0	0	1.8±0.03 (19±0.03) [91±1]	0.2	-	1.6±0.04 (16±0.04) [80±1]	2.5	-	11.5±0.2 (16±0.2) [80±1]	1.7	-	N.D.	-	-
155		4	8	8	8	5.9±0.1	1.7	103±3	9.8±0.2	2.0	103±3	19.7±0.2	1.0	103±3	7.8±0.1	1.3	98±2
156		8	15	15	15	10.3±0.1	1.0	106±2	17.1±0.2	1.2	103±2	27.3±0.2	0.7	105±2	13.7±0.1	0.7	91±1

Table 13 (cont.)

No.	Sample	Added ($mg\ L^{-1}$)				Concentration found, % relative standard deviation and % recovery (n=3)											
						GA			C			EC			EGCG		
		GA	C	EC	EGCG	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec	$mg\ L^{-1}$ [$mg\ kg^{-1}$]	%RSD	%Rec
157	BR17	0	0	0	0	N.D.	-	-	N.D.	-	-	13.1±0.1 (263±0.1) [1285±2]	0.8	-	N.D.	-	-
158		4	8	8	8	3.4±0.03	0.9	85±1	6.8±0.1	1.5	85±1	21.2±0.2	0.9	101±3	N.D.	-	-
159		8	15	15	15	5.6±0.1	1.8	70±1	13.6±0.1	0.7	91±1	28.8±0.1	0.4	105±1	N.D.	-	-
160	BR18	0	0	0	0	N.D.	-	-	N.D.	-	-	10.0±0.2 (400±0.2) [1877±4]	2.0	-	N.D.	-	-
161		4	8	8	8	4.3±0.03	0.7	108±1	7.9±0.4	5.1	99±5	18.0±0.4	2.2	100±5	6.3±0.1	1.6	79±5
162		8	15	15	15	8.1±0.2	2.4	101±2	15.6±0.3	1.9	104±4	24.9±0.4	1.6	99±3	12.0±0.4	3.3	80±3

[a] P1 – P9 referred to raw peel samples of banana crude extracts, PR10 – PR18 referred to ripe peel samples of banana crude extracts, H1 – H9 referred to raw hand stalk samples of banana crude extracts, HR10 – HR18 referred to ripe hand stalk samples of banana crude extracts, B1 – B9 referred to raw bunch stalk samples of banana crude extracts, BR10 – BR18 referred to ripe bunch stalk samples of banana crude extracts, [b] Average value ± standard deviation of triplicate results ($\overline{X}$±SD), [c] Concentrations were found by comparing the calibration graph of each analyte, [d] Actual concentrations were obtained by calculating dilution factor of each sample, [e] Actual concentrations were obtained by calculating milligram per kilogram of each dried crude extract, and [f] N.D. – not detected (or < LOD)

Table 14 Total concentrations found of GA, C, EC and EGCG in banana crude extracts by the proposed HPLC

Banana crude extracts	Abbreviations	Total concentrations found (mg kg^{-1} of banana crude extracts)			
		GA	C	EC	EGCG
Raw peel	P1 – P9	349±4[a]	489±5	1359±10	48±0.3
Ripe peel	PR10 – PR18	788±4	111±3	1171±13	23±1
Raw hand stalk	H1 – H9	337±3	3658±13	6003±33	275±3
Ripe hand stalk	HR10 – HR18	340±5	903±8	4648±12	N.D.[b]
Raw bunch stalk	B1 – B9	1387±4	7247±17	4896±16	190±2
Ripe bunch stalk	BR10 - BR18	767±7	282±6	8206±38	N.D.

[a] $\bar{X}$±SD - Mean ± standard deviation, [b] N.D. - Not detected (or < LOD)

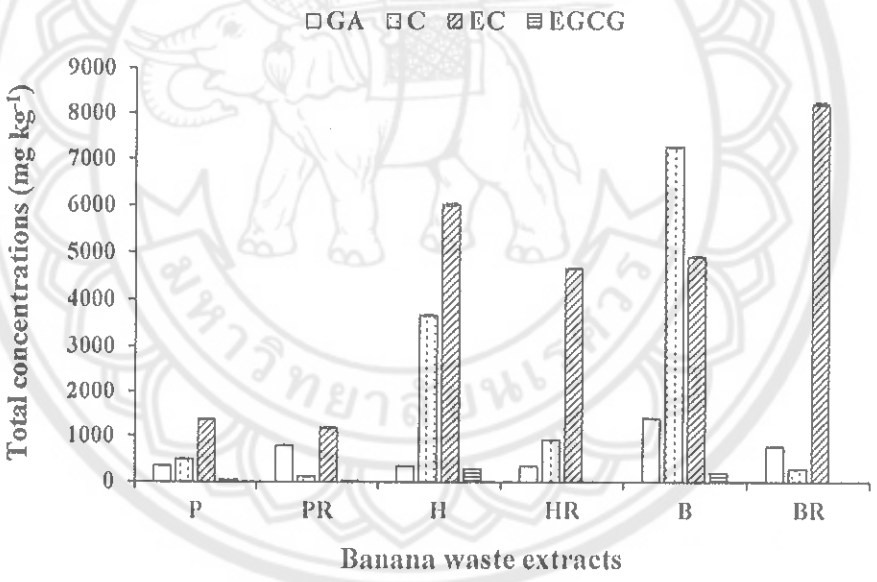


Figure 13 Total concentrations found of GA, C, EC and EGCG in banana crude extracts by the proposed HPLC method; peel (P), ripe peel (PR), raw hand stalk (H), ripe hand stalk (HR), raw bunch stalk (B) and ripe bunch stalk (BR)

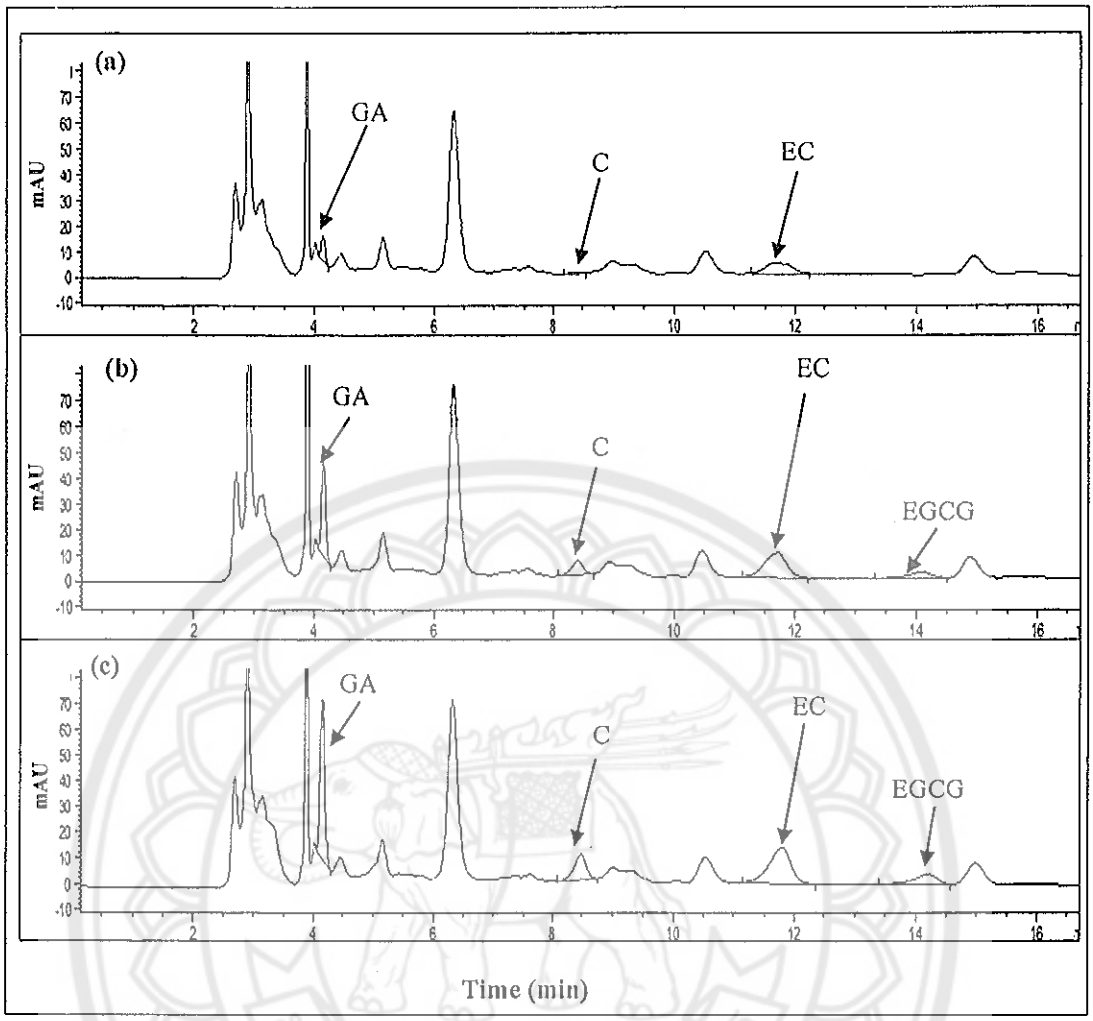


Figure 14 HPLC chromatograms for the determination of: (a) a banana crude extract sample of ripe bunch stalk from Phitsanulok province in rainy season, (b) sample + low concentrations of a mixed standard solution (4 mg L^{-1} of GA and 8 mg L^{-1} of GC, C, EC and EGCG, respectively) and (c) sample + high concentration of a mixed standard solution (8 mg L^{-1} of GA and 15 mg L^{-1} of GC, C, EC and EGCG, respectively)

2. The chromatographic fingerprint analysis

To achieve the chromatographic fingerprint pattern of the 54 samples of banana crude extracts. The HPLC data of all samples such as raw chromatogram patterns and retention times were used in the analysis of chromatographic fingerprint procedures. These HPLC data processing were overlapped all chromatograms, normalized retention times, and then selected peak markers. Results are shown in Table 15 - 17 and Figure

15. It was found that six peak markers were selected as the representative of peels, hand stalks and bunch stalks. Peak 4 was an epicatechin (EC) peak and was assigned as the reference peak. The RSDs of the RRT were in the range 0.5 – 3.7, 0.8 – 4.5, and 1.0 – 3.4 of peels, hand stalks and bunch stalks. These %RSD values indicated that each all peak markers were precise (%RSD <5). The RTs of the peak markers of peels were 4.67 ± 0.17 (peak 1), 5.85 ± 0.21 (peak 2), 10.03 ± 0.18 (peak 3), 11.30 ± 0.06 (peak 4; EC), and 14.50 ± 0.13 (peak 5) and 17.55 ± 0.14 (peak 6) which was similar to hand stalks. And, the RTs of peak markers of bunch stalks were 4.67 ± 0.17 (peak 1), 5.85 ± 0.21 (peak 2), 10.03 ± 0.18 (peak 3), 11.30 ± 0.06 (peak 4; EC), 14.50 ± 0.13 (peak 5) and 22.83 ± 0.06 (peak 6). The proposed combination of the quantitative and chromatographic fingerprint analyses has been successfully applied for the quantity and quality profiles of antioxidants in samples of banana crude extracted.

Table 15 Retention times (RT) and relative retention times (RRT) of six characteristic peak markers of peel banana extract samples (both raws and ripes)

Sample	Peak 1		Peak 2		Peak 3		Peak 4 (EC)		Peak 5		Peak 6	
	RT	RRT a	RT	RRT	RT	RRT	RT	RRT	RT	RRT	RT	RRT
P1	4.49	2.51	6.03	1.87	9.95	1.13	11.26	1	14.21	0.79	17.19	0.65
P2	4.85	2.31	5.91	1.90	9.95	1.13	11.21	1	14.25	0.79	17.52	0.64
P3	4.75	2.37	5.78	1.94	9.90	1.14	11.24	1	14.36	0.78	17.60	0.64
P4	4.72	2.39	5.81	1.94	9.93	1.13	11.26	1	14.34	0.79	17.51	0.64
P5	4.57	2.46	5.74	1.95	9.83	1.14	11.22	1	14.32	0.78	17.45	0.64
P6	4.71	2.41	5.83	1.95	9.99	1.14	11.35	1	14.50	0.78	17.67	0.64
P7	4.67	2.43	5.83	1.94	9.92	1.14	11.32	1	14.43	0.78	17.50	0.65
P8	4.77	2.38	5.90	1.92	10.02	1.13	11.35	1	14.40	0.79	17.41	0.65
P9	4.26	2.65	5.69	1.98	9.95	1.13	11.27	1	14.55	0.77	17.64	0.64
PR10	4.61	2.45	5.71	1.98	9.89	1.14	11.32	1	14.46	0.78	17.69	0.64
PR11	4.93	2.29	6.04	1.87	9.95	1.13	11.29	1	14.29	0.79	17.39	0.65
PR12	4.65	2.43	5.71	1.97	9.89	1.14	11.28	1	14.42	0.78	17.79	0.63
PR13	4.52	2.50	5.99	1.89	9.93	1.14	11.31	1	14.45	0.78	17.63	0.64
PR14	4.66	2.42	5.78	1.95	9.97	1.13	11.29	1	14.49	0.78	17.64	0.64
PR15	4.67	2.43	5.84	1.94	9.97	1.14	11.33	1	14.43	0.79	17.50	0.65
PR16	4.43	2.56	5.61	2.02	9.89	1.15	11.33	1	14.55	0.78	17.57	0.64

Table 15 (cont.)

Sample	Peak 1		Peak 2		Peak 3		Peak 4 (EC)		Peak 5		Peak 6	
	RT	RRT [a]	RT	RRT	RT	RRT	RT	RRT	RT	RRT	RT	RRT
PR17	4.91	2.34	6.10	1.88	10.2	1.13	11.49	1	14.52	0.79	17.51	0.66
PR18	4.46	2.54	5.73	1.97	9.90	1.14	11.32	1	14.53	0.78	17.64	0.64
% RSD of RRT [b]	-	3.7	-	2.2	-	0.5	-	0	-	0.6	-	0.9

[a] RRT- Relative retention time was calculated by RT of characteristic peak / RT of epicatechin (EC) reference peak.

[b] Percentage relative standard deviation (n=3)

Table 16 Retention times (RT) and relative retention times (RRT) of six characteristic peak markers of hand stalk banana extract samples (both raws and ripes)

Sample	Peak 1		Peak 2		Peak 3		Peak 4 (EC)		Peak 5		Peak 6	
	RT	RRT [a]	RT	RRT	RT	RRT	RT	RRT	RT	RRT	RT	RRT
H1	4.51	2.53	5.83	1.96	9.97	1.15	11.43	1	14.58	0.78	17.54	0.65
H2	4.81	2.37	5.97	1.91	9.97	1.15	11.42	1	14.38	0.79	17.52	0.65
H3	4.74	2.40	5.82	1.95	10.01	1.14	11.37	1	14.42	0.79	17.50	0.65
H4	4.62	2.45	5.82	1.95	9.97	1.14	11.34	1	14.36	0.79	17.58	0.65
H5	4.64	2.46	5.91	1.93	10.08	1.13	11.42	1	14.62	0.78	17.80	0.64
H6	4.53	2.49	5.82	1.94	10.19	1.11	11.31	1	14.45	0.78	17.50	0.65
H7	4.74	2.41	5.91	1.93	10.07	1.13	11.42	1	14.53	0.79	17.58	0.65
H8	4.75	2.38	5.96	1.90	10.08	1.12	11.33	1	14.44	0.78	17.48	0.65
H9	4.60	2.46	6.03	1.88	10.06	1.13	11.33	1	14.50	0.78	17.60	0.64
HR10	4.49	2.52	5.57	2.03	9.945	1.14	11.31	1	14.89	0.78	17.42	0.65
HR11	5.13	2.23	6.25	1.84	10.14	1.13	11.47	1	14.38	0.80	17.38	0.66
HR12	4.43	2.57	5.73	1.99	9.97	1.14	11.39	1	14.51	0.78	17.70	0.64
HR13	4.44	2.51	5.36	2.08	9.77	1.14	11.13	1	14.29	0.78	17.40	0.64
HR14	4.68	2.40	5.83	1.93	9.99	1.13	11.25	1	14.58	0.77	17.81	0.63
HR15	4.42	2.56	5.21	2.17	9.86	1.14	11.29	1	14.52	0.78	17.70	0.64
HR16	4.67	2.39	5.19	2.16	9.52	1.17	11.18	1	14.42	0.78	17.61	0.63
HR17	4.68	2.41	5.85	1.93	10.05	1.12	11.29	1	14.46	0.78	17.55	0.64
HR18	4.66	2.44	5.87	1.94	9.99	1.14	11.40	1	14.52	0.78	17.60	0.65
% RSD of RRT [b]	-	3.3	-	4.5	-	1.2	-	0	-	0.8	-	1.0

[a] RRT- Relative retention time was calculated by RT of characteristic peak / RT of epicatechin (EC) reference peak

[b] Percentage relative standard deviation (n=3)

Table 17 Retention times (RT) and relative retention times (RRT) of six characteristic peak markers of bunch stalk banana extract samples (both raws and ripes)

Sample	Peak 1		Peak 2		Peak 3		Peak 4 (EC)		Peak 5		Peak 6	
	RT	RRT [a]	RT	RRT	RT	RRT	RT	RRT	RT	RRT	RT	RRT
B1	4.68	2.45	5.82	1.97	10.41	1.10	11.50	1	14.63	0.79	22.82	0.50
B2	4.64	2.45	5.76	1.97	10.30	1.10	11.34	1	14.46	0.78	22.36	0.51
B3	4.67	2.44	5.86	1.95	10.38	1.10	11.40	1	14.63	0.78	22.85	0.50
B4	4.72	2.42	5.95	1.92	10.51	1.09	11.44	1	14.64	0.78	22.96	0.50
B5	4.75	2.45	5.93	1.96	10.31	1.13	11.63	1	14.66	0.79	23.07	0.50
B6	4.64	2.45	5.86	1.94	10.21	1.11	11.35	1	14.46	0.79	22.78	0.50
B7	4.23	2.66	5.63	2.00	9.89	1.14	11.23	1	14.65	0.77	23.33	0.48
B8	4.66	2.42	5.84	1.94	10.04	1.13	11.30	1	14.46	0.78	22.74	0.50
B9	4.68	2.40	5.93	1.89	10.30	1.09	11.22	1	14.62	0.77	22.97	0.49
BR10	4.61	2.48	5.84	1.96	10.09	1.13	11.43	1	14.76	0.77	22.96	0.50
BR11	5.07	2.27	6.16	1.87	10.18	1.13	11.49	1	14.46	0.79	22.32	0.51
BR12	4.80	2.37	6.32	1.80	9.85	1.15	11.36	1	14.68	0.77	22.66	0.50
BR13	4.75	2.38	5.89	1.92	9.85	1.15	11.30	1	14.57	0.78	22.98	0.49
BR14	4.63	2.47	5.94	1.93	10.07	1.14	11.45	1	14.63	0.78	22.76	0.50
BR15	4.77	2.41	5.94	1.93	10.09	1.14	11.48	1	14.55	0.79	22.74	0.50
BR16	4.68	2.45	5.97	1.92	10.18	1.12	11.44	1	14.65	0.78	23.43	0.49
BR17	4.97	2.29	6.10	1.86	10.27	1.11	11.36	1	14.69	0.77	22.92	0.50
BR18	4.87	2.35	6.16	1.86	10.27	1.11	11.43	1	14.85	0.77	22.35	0.51
% RSD of RRT [b]	-	3.4	-	2.6	-	1.7	-	0	-	1.0	-	1.7

[a] RRT- Relative retention time was calculated by RT of characteristic peak / RT of epicatechin (EC) reference peak.

[b] Percentage relative standard deviation (n=3)

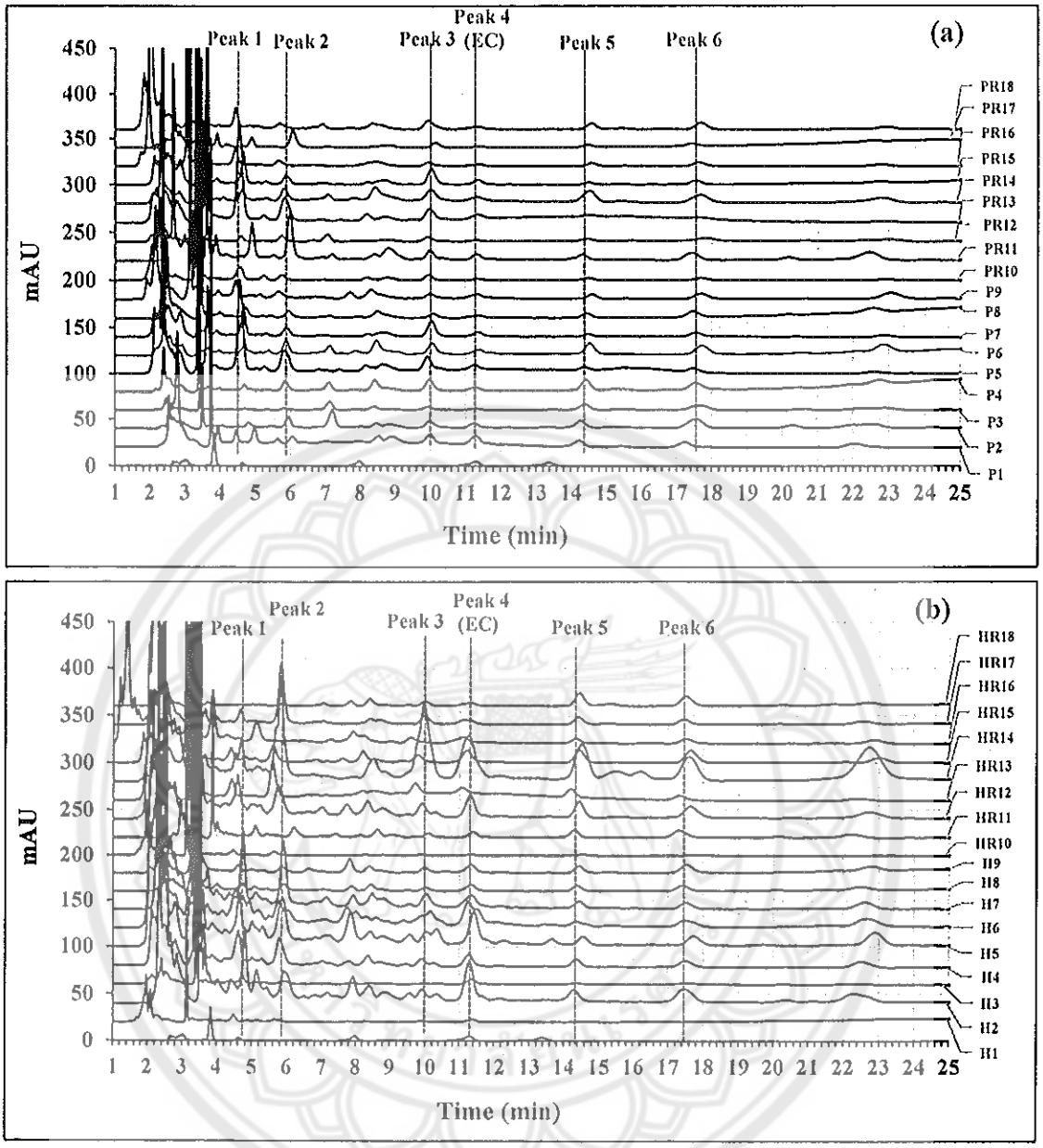


Figure 15 Chromatographic fingerprint patterns of banana extract samples: (a) peels, (b) hand stalks and (c) bunch stalks

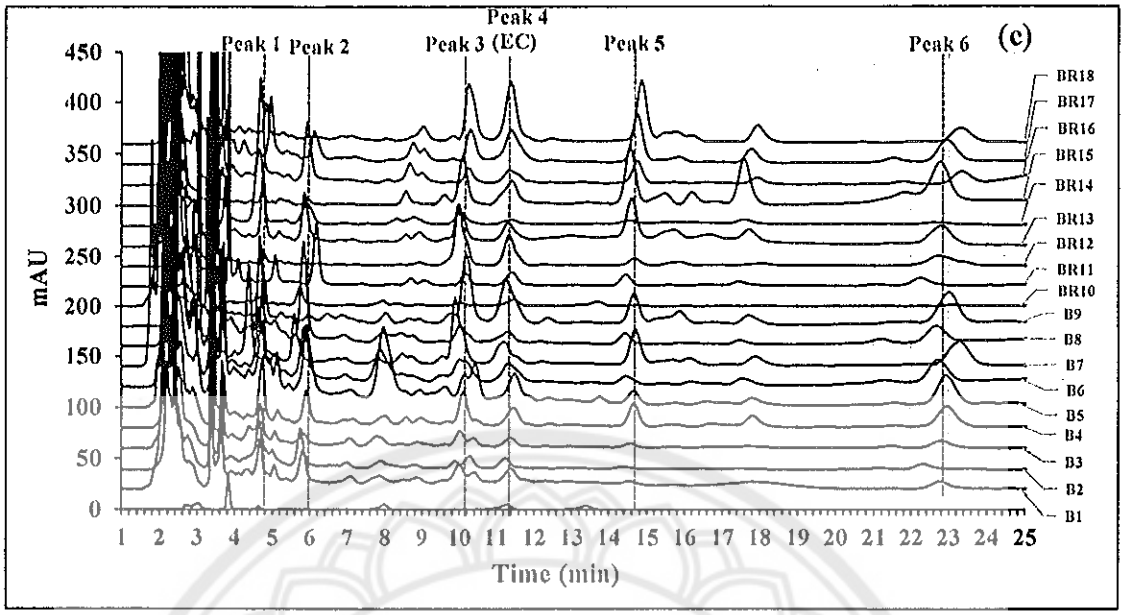


Figure 15 (cont.)

Determination of antioxidant capacity using ABTS assay and total phenolic compounds using FC assay in teas and herbal teas by continuous flow injection systems

1. Study of absorption spectra of ABTS and FC assays

The absorption spectra were preliminary studied the reactions of a gallic acid (GA) with a ABTS radical cation ($ABTS^{\bullet+}$) and a GA with a folin-ciocalteu's phenol (FC) at pH ~10, prior to determine antioxidant capacity and total phenolic compounds by continuous flow injection systems. The study was performed by using a UV/Vis spectrophotometer to obtain absorption spectra and maximum wavelengths of solutions. Results are shown in Figure 16(a) and 16(b). For ABTS assay, it was found that the maximum wavelength of ABTS was at 314 nm while $K_2S_2O_8$ was unseen at 300 - 900 nm. The $ABTS^{\bullet+}$ was appeared the maximum wavelengths at 417, 645, 730 and 826 nm and these maximum wavelengths of $ABTS^{\bullet+}$ + GA reaction were similar (decreasing a blue-green color intensity). Thus, the maximum wavelength at 730 were selected.

For FC assay, the increase in blue color intensity of FC solution was measured when the GA and NaOH solutions were added. The FC and NaOH solutions were invisible between 500 – 900 nm and 250 – 900 nm, respectively. The maximum wavelengths of FC + GA and FC + GA + NaOH were similar at 743 nm but absorption

intensity of FC+GA+NaOH was higher than FC+GA. Therefore, the detection wavelength at 728 nm was chosen for the determination of total phenolic compounds.

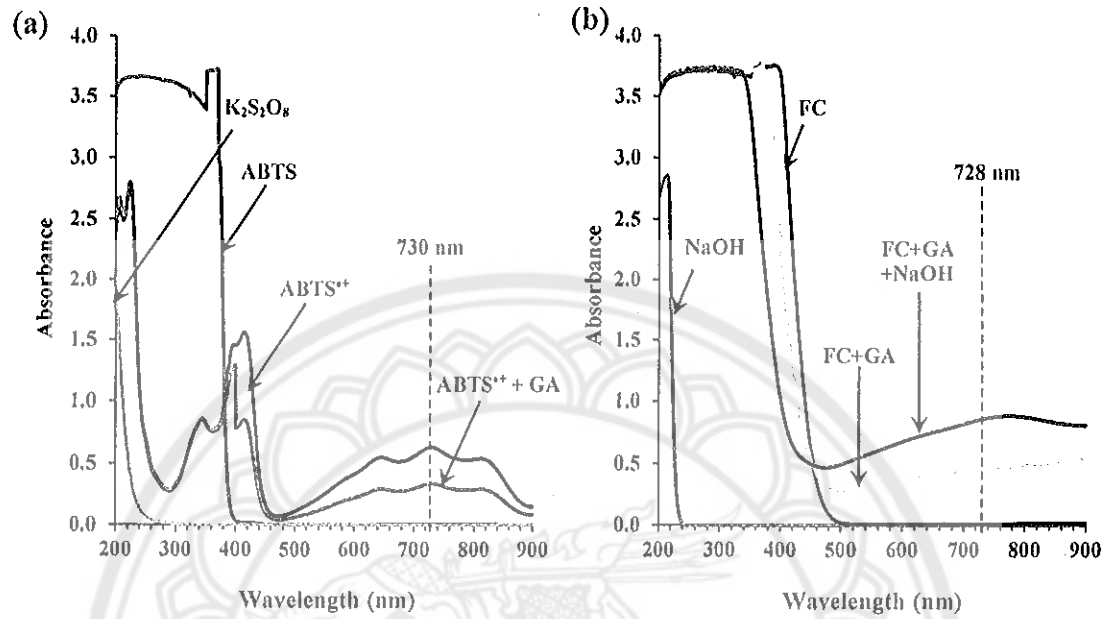


Figure 16 Absorption spectra of solutions of: (a) ABTS (7 mmol L^{-1}), $K_2S_2O_8$ (2.45 mmol L^{-1}), $ABTS^{\bullet+}$ (1:85 v/v of $ABTS^{\bullet+}$:H_2O), and $ABTS^{\bullet+}$ + GA (0.01 mol L^{-1}) solutions, and (b) FC (1:10 v/v of FC:H_2O), NaOH (0.20 mol L^{-1}), FC + GA (1.0 mmol L^{-1}) and FC + GA + NaOH solutions

2. Optimization of the continuous flow systems using ABTS and FC assays

Preliminary conditions of the continuous flow systems using ABTS and FC assays in Figure 7 for the determination of antioxidant capacity and total phenolic compounds, respectively, were used as shown in Table 18. Various preliminary parameters were optimized as following.

Table 18 Preliminary conditions used of the continuous flow systems using ABTS and FC assays for the determination antioxidant capacity and total phenolic compounds, respectively

Parameters	Conditions used	
	ABTS assay	FC assay
Continuous flow system:		
Carrier solution (C_s)	Water	Water
Standard/sample (S)	Gallic acid	Gallic acid
Reagent solutions: R_A	$ABTS^{\bullet+}$ in water (expressed as 0.7 absorbance)	-
R_F		FC:water (1:10)
R_N		NaOH (0.25 mol L^{-1})
Flow rate	1.0 mL min^{-1}	1.6 mL min^{-1}
Reaction loop (RL) length	20 cm	50 cm
Volumes of S:R_A and S:R_F:R_N	17 µL: 17 µL (equal to 1s and 1s of aspiration times)	27 µL: 27 µL: 27 µL (equal to 1s, 1s and 1s of aspiration times)
Stopped time at RL	30 s	0 s
Detection wavelength	730 nm	728 nm
Operation times of the system:		
1) Filling steps of:		
- C_s to RL, FTC and W	120 s	130 s
- S to RL, WC and W	65 s	40 s
- R_A to RL, WC and W	65 s	-
- R_F to RL, WC and W	-	45 s
- R_N to RL, WC and W	-	42 s
- C_s to RL, WC and W	160 s	120 s
2) Loading steps of:		
- Rapid sequenced aspiration of S and R_A at RL to WC and W	20 s (S:R_A = 1s:1s)	-
- Rapid sequenced aspiration of S, R_F and R_N at RL to WC and W	-	30 s (S:R_F:R_N = 1s:1s:1s)
3) Injection and cleaning steps of:		
- S + R_A zone at RL to FC and W	250 s	-
- S + R_F + R_N zone at RL to FC and W	-	250 s

Table 18 (cont.)

Parameters	Conditions used	
	ABTS assay	FC assay
Operation times of the system:		
4) Cleaning steps of:		
- C_s to RL, FTC and W	250 s	250 s
- S to RL, WC and W	80 s	90 s
- R_A to WC and W	80 s	-
- R_F to RL, WC and W	-	90 s
- R_N to RL, WC and W	-	90 s
- C_s to RL, WC and W	160 s	150 s

2.1 ABTS assay

2.1.1 Effect of types of carrier solution

A carrier solution (C_s) in this work is a solution for transport the S + R_A zone toward the detector. It may affect to sensitivity and stability of $ABTS^{\bullet+}$. Thus, types of carrier solution were examined to obtain high sensitivity and to study the stability of $ABTS^{\bullet+}$ solution. Two solutions of water and acetate buffer (0.02 mol L^{-1}, pH 4.5) were optimized under the preliminary conditions in Table 18. Blank (water) and 1.0 mg L^{-1} GA were aspirated into the system for 12 hours. Results were provided in Table 19 and Figure 17. It was found that both solutions were no different in the stability of $ABTS^{\bullet+}$ solution within 12 hours, although acetate buffer was slightly higher peak height (sensitivity). With a reasonable stability and sensitivity, the acetate buffer was selected for further study using ABTS assay.

Table 19 Effect of types of carrier solution on peak height of GA standard solutions

Carrier solution	Time (h)	GA (mg L^{-1})	Peak height (mV)					
			1	2	3	$\overline{X}$	Blank- $\overline{X}$	SD
Water	0	0 (blank)	0.859	0.839	0.839	0.846	0.000	0.012
		1.0	0.430	0.449	0.430	0.436	0.410	0.011
	1	0 (blank)	0.859	0.840	0.840	0.846	0.000	0.011
		1.0	0.430	0.449	0.449	0.443	0.404	0.011
	2	0 (blank)	0.840	0.840	0.859	0.846	0.000	0.011
		1.0	0.449	0.469	0.469	0.462	0.384	0.011

Table 19 (cont.)

Carrier solution	Time (h)	GA (mg L^{-1})	Peak height (mV)					
			1	2	3	$\overline{X}$	Blank- $\overline{X}$	SD
	3	0 (blank)	0.859	0.840	0.859	0.853	0.000	0.011
		1.0	0.449	0.449	0.449	0.449	0.404	0.000
	4	0 (blank)	0.840	0.840	0.857	0.846	0.000	0.010
		1.0	0.447	0.447	0.447	0.447	0.399	0.000
	5	0 (blank)	0.820	0.840	0.840	0.833	0.000	0.011
		1.0	0.449	0.449	0.449	0.449	0.384	0.000
	6	0 (blank)	0.840	0.840	0.840	0.840	0.000	0.000
		1.0	0.430	0.449	0.430	0.436	0.404	0.011
	7	0 (blank)	0.820	0.840	0.840	0.840	0.000	0.011
		1.0	0.430	0.449	0.449	0.443	0.391	0.011
	8	0 (blank)	0.840	0.840	0.840	0.840	0.000	0.000
		1.0	0.430	0.449	0.449	0.443	0.397	0.011
	9	0 (blank)	0.840	0.820	0.840	0.840	0.000	0.011
		1.0	0.430	0.449	0.449	0.443	0.391	0.011
	10	0 (blank)	0.820	0.840	0.840	0.833	0.000	0.011
		1.0	0.449	0.469	0.469	0.462	0.371	0.011
	12	0 (blank)	0.840	0.840	0.858	0.846	0.000	0.010
		1.0	0.488	0.488	0.486	0.488	0.358	0.001
Acetate buffer (pH 4.5)	0	0 (blank)	0.781	0.801	0.799	0.794	0.000	0.022
		1.0	0.508	0.508	0.580	0.508	0.286	0.022
	1	0 (blank)	0.801	0.801	0.801	0.801	0.000	0.000
		1.0	0.488	0.488	0.488	0.488	0.313	0.000
	2	0 (blank)	0.781	0.801	0.820	0.801	0.000	0.000
		1.0	0.488	0.488	0.488	0.488	0.313	0.000
	3	0 (blank)	0.801	0.801	0.801	0.801	0.000	0.000
		1.0	0.469	0.469	0.488	0.475	0.326	0.010
	4	0 (blank)	0.801	0.801	0.781	0.794	0.000	0.010
		1.0	0.469	0.486	0.486	0.480	0.314	0.014
	5	0 (blank)	0.801	0.801	0.801	0.801	0.000	0.000
		1.0	0.488	0.486	0.487	0.487	0.314	0.019
	6	0 (blank)	0.781	0.781	0.781	0.781	0.000	0.000
		1.0	0.449	0.467	0.486	0.467	0.314	0.019
	7	0 (blank)	0.781	0.801	0.801	0.794	0.000	0.010
		1.0	0.469	0.488	0.488	0.482	0.313	0.014
	8	0 (blank)	0.781	0.781	0.781	0.781	0.000	0.000
		1.0	0.488	0.488	0.508	0.495	0.286	0.011
	9	0 (blank)	0.801	0.820	0.820	0.814	0.000	0.010
		1.0	0.508	0.808	0.527	0.514	0.299	0.015
	10	0 (blank)	0.801	0.801	0.801	0.801	0.000	0.000
		1.0	0.508	0.508	0.508	0.508	0.293	0.000
	12	0 (blank)	0.762	0.781	0.781	0.775	0.000	0.010
		1.0	0.469	0.488	0.484	0.480	0.297	0.013

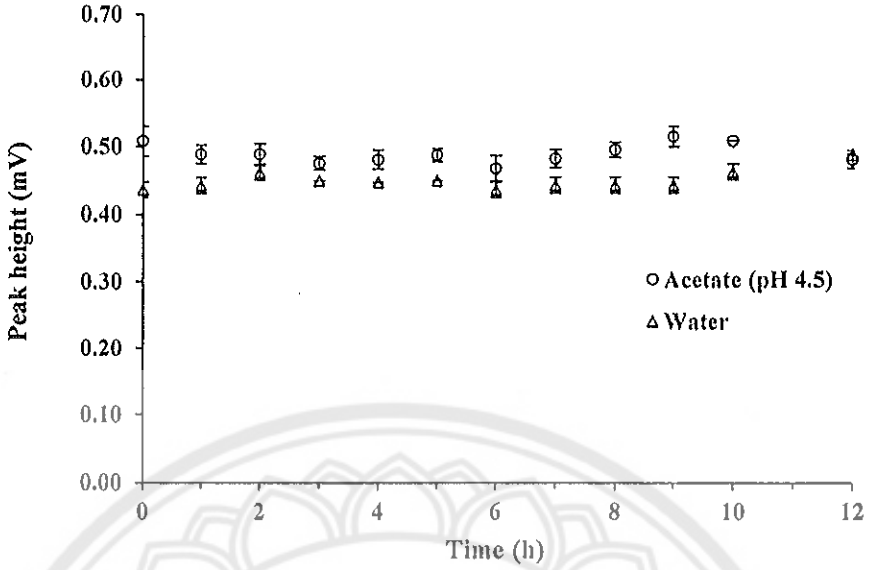


Figure 17 Types of carrier solution on peak height of GA (1.0 mg L^{-1}) at the different the detection times

2.2.2 Effect of $ABTS^{\bullet+}$ concentrations

The concentrations of $ABTS^{\bullet+}$ working solution is affected to linear range, linearity, sensitivity and analysis time. Thus, the effect of $ABTS^{\bullet+}$ concentrations was studied to obtain wide linear range of GA, high sensitivity, and acceptable analysis time. The $ABTS^{\bullet+}$ concentration (expressed as absorbance intensity; A) was optimized in the range of 0.65 – 0.95 A by aspiration blank (water) and GA (0.1, 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 mg L^{-1}) into the system under the preliminary conditions in Table 18 and the obtained condition in 2.1.1. The results in Table 20 and Figure 18 showed that the increasing absorbance intensity gave the wide linear range, high linearity and long analysis time while the sensitivity (slope) decreased. Thus, to obtain wide linear range (0.25 – 2.50 mg L^{-1} GA), good linearity (r^2 = 0.9963), good sensitivity (slope = 0.7325) and acceptable analysis time (5 min), the absorbance intensity at 0.84 A was selected for the next study.

Table 20 Effect of $ABTS^{\bullet+}$ concentrations (expressed as an absorbance intensity; A) on peak height and slope of GA standard solutions

$ABTS^{\bullet+}$ (A)	GA (mg L^{-1})	Peak height (mV)						Slope	r^2
		1	2	3	$\overline{X}$	Blank- $\overline{X}$	SD		
0.65	0 (blank)	1.738	1.680	1.660	1.693	0.000	0.041	0.7647 ± 0.0347	0.9925
	0.25	1.536	1.536	1.502	1.525	0.168	0.020		
	0.50	1.402	1.447	1.402	1.417	0.276	0.026		
	1.0	0.999	0.999	0.999	0.999	0.694	0.000		
	1.5	0.506	0.551	0.551	0.536	1.157	0.026		
	2.0	0.193	0.193	0.193	0.193	1.500	0.000		
0.75	0 (blank)	1.973	1.992	2.012	1.992	0.000	0.020	0.7253 ± 0.0272	0.9942
	0.25	1.844	1.844	1.844	1.844	0.148	0.020		
	0.50	1.670	1.670	1.670	1.670	0.322	0.000		
	1.0	1.191	1.235	1.235	1.220	0.772	0.000		
	1.5	0.843	0.843	0.843	0.843	1.149	0.025		
	2.0	0.539	0.539	0.539	0.539	1.453	0.000		
	2.5	0.234	0.234	0.234	0.234	1.758	0.000		
0.84	0 (blank)	2.070	2.109	2.109	2.096	0.000	0.023	0.7325 ± 0.0060	0.9963
	0.25	1.925	1.925	1.967	1.939	0.157	0.024		
	0.50	1.842	1.883	1.883	1.869	0.227	0.024		
	1.0	1.383	1.424	1.424	1.411	0.685	0.024		
	1.5	1.049	1.049	1.049	1.049	1.047	0.000		
	2.0	0.674	0.715	0.715	0.701	1.395	0.024		
	2.5	0.340	0.340	0.340	0.340	1.756	0.000		
0.95	0 (blank)	2.422	2.324	2.324	2.357	0.000	0.056	0.7156 ± 0.0335	0.9919
	0.25	2.168	2.128	2.128	2.141	0.216	0.023		
	0.50	2.042	2.085	2.085	2.071	0.286	0.025		
	1.0	1.613	1.608	1.570	1.597	0.760	0.023		
	1.5	1.227	1.184	1.184	1.198	1.159	0.025		
	2.0	0.927	0.970	0.970	0.955	1.402	0.025		
	2.5	0.541	0.584	0.584	0.569	1.788	0.025		

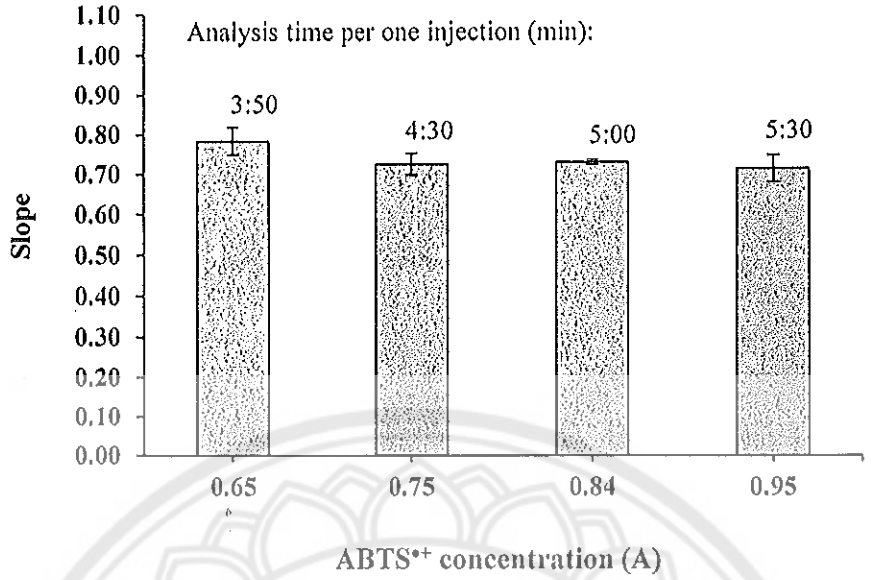


Figure 18 Effect of $ABTS^{\bullet+}$ concentrations (expressed as an absorbance (A)) on slope of GA (0.25 – 2.50 mg L^{-1})

2.1.3 Effect of flow rates

A flow rate is affecting to the dispersion of a continuous flow system involved in the sensitivity, characteristic peak and analysis time of the measurement. In order to obtain high sensitivity, sharp peak and short analysis time, the effect of flow rates was studied in the range of 1.0 – 2.0 mL min^{-1}. Using the preliminary conditions in Table 18 and obtained conditions in 2.1.1 to 2.1.2, a blank (water) and GA (0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 mg L^{-1}) were aspirated into the system. Results are shown in Table 21 and Figure 19. A flow rate of 1.0 mL min^{-1} showed the highest sensitivity but it gave tailing peak and long analysis time (5 min). A flow rate at 2.0 mL min^{-1} was resulted a short analysis time, sharp peak but low sensitivity was observed because of high dispersion. Therefore, a flow rate of 1.6 mL min^{-1} was selected due to the best compromise of the sensitivity, characteristic peak and analysis time (2 min 30 sec).

Table 21 Effect of flow rates on peak height and slope of GA standard solutions

Flow rate (mL min^{-1})	GA (mg L^{-1})	Peak height (mV)						Slope	r^2
		1	2	3	$\overline{X}$	Blank- $\overline{X}$	SD		
1.0	0 (blank)	2.168	2.168	2.129	2.155	0.000	0.023	0.7120 ± 0.0078	0.9995
	0.25	2.014	2.014	1.975	2.001	0.154	0.023		
	0.50	1.815	1.775	1.776	1.789	0.366	0.023		
	1.0	1.456	1.456	1.457	1.457	0.698	0.000		
	1.5	1.058	1.098	1.098	1.084	1.071	0.023		
	2.0	0.739	0.739	0.779	0.752	1.403	0.023		
	2.5	0.380	0.380	0.381	0.380	1.775	0.000		
1.6	0 (blank)	2.109	2.110	2.100	2.107	0.000	0.005	0.6746 ± 0.0092	0.9989
	0.25	1.925	1.926	1.916	1.922	0.184	0.005		
	0.50	1.740	1.777	1.768	1.762	0.345	0.020		
	1.0	1.443	1.518	1.434	1.465	0.642	0.046		
	1.5	1.109	1.072	1.100	1.094	1.013	0.019		
	2.0	0.738	0.775	0.803	0.772	1.334	0.033		
	2.5	0.404	0.404	0.395	0.401	1.706	0.005		
2.0	0 (blank)	1.992	1.992	2.012	1.999	0.000	0.011	0.6524 ± 0.0221	0.9938
	0.25	1.855	1.798	1.798	1.817	1.82	0.033		
	0.50	1.630	1.648	1.648	1.642	0.357	0.010		
	1.0	1.331	1.312	1.312	1.318	0.680	0.011		
	1.5	0.995	0.975	0.975	0.982	1.017	0.011		
	2.0	0.621	0.564	0.564	0.583	1.416	0.033		
	2.5	0.397	0.415	0.377	0.396	1.603	0.019		

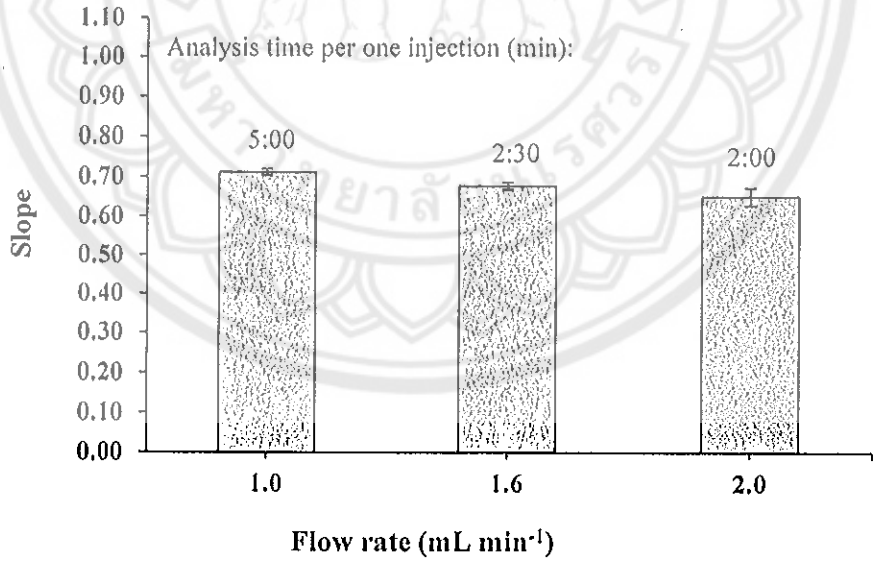


Figure 19 Effect of flow rates on slope of GA (0.25 – 2.5 mg L^{-1})

2.1.4 Effect of stopped times at reaction loop

It has been known that dispersion of the S + R_A zone at reaction loop affected to sensitivity and analysis time. To increase the sensitivity (slope) of GA + $ABTS^{\bullet+}$ reaction, the effect of stopped times (0, 10, 20, 30, 40, 50, and 60 s) at reaction loop (RL) was investigated. A Blank and GA (0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 mg L^{-1}) were aspirated into the system using preliminary conditions in Table 18 and obtained conditions in 2.1.1 to 2.1.3. Results are shown in Table 22 and Figure 20. It was certain that the sensitivity increased when the stopped times increased, including analysis time per one injection. Thus, a stopped time of 30 sec was selected because of good sensitivity and analysis time (2 min 30 sec).

Table 22 Effect of stopped times at reaction loop on peak height and slope of GA standard solutions

Stopped time (s)	GA (mg L^{-1})	Peak height (mV)						Slope	r^2
		1	2	3	$\overline{X}$	Blank- $\overline{X}$	SD		
0	0 (blank)	2.070	2.051	2.051	2.057	0.000	0.016	0.6553 ± 0.0089	0.9993
	0.25	1.973	1.992	1.992	1.986	0.119	0.010		
	0.50	1.797	1.855	1.855	1.836	0.269	0.029		
	1.0	1.543	1.543	1.523	1.536	0.568	0.011		
	1.5	1.172	1.191	1.211	1.191	0.913	0.017		
	2.0	0.859	0.879	0.840	0.859	1.245	0.019		
	2.5	0.508	0.527	0.508	0.514	1.590	0.011		
10	0 (blank)	2.090	2.109	2.148	2.116	0.000	0.033	0.7017 ± 0.0160	0.9979
	0.25	1.992	1.992	1.992	1.992	0.059	0.000		
	0.50	1.836	1.816	1.836	1.829	0.221	0.011		
	1.0	1.484	1.465	1.465	1.471	0.579	0.025		
	1.5	1.191	1.094	1.094	1.126	0.925	0.046		
	2.0	0.684	0.723	0.742	0.716	1.335	0.030		
	2.5	0.449	0.449	0.449	0.449	1.602	0.010		
20	0 (blank)	2.637	2.637	2.656	2.643	0.000	0.011	0.7011 ± 0.0090	0.9992
	0.25	1.993	2.066	2.049	2.036	0.607	0.038		
	0.50	1.846	1.846	.829	1.840	0.803	0.010		
	1.0	1.442	1.479	1.462	1.461	1.182	0.018		
	1.5	1.149	1.185	1.132	1.155	1.488	0.028		
	2.0	0.782	0.819	0.765	0.788	1.855	0.028		
	2.5	0.452	0.452	0.434	0.446	2.197	0.010		
30	0 (blank)	2.207	2.168	2.168	2.181	0.000	0.023	0.7255 ± 0.0140	0.9979
	0.25	2.025	1.987	2.063	2.025	0.156	0.038		
	0.50	1.834	1.834	1.834	1.834	0.347	0.000		
	1.0	1.492	1.492	1.492	1.492	0.689	0.000		
	1.5	1.111	1.073	1.111	1.098	1.083	0.022		
	2.0	0.769	0.807	0.845	0.807	1.374	0.038		
	2.5	0.388	0.312	0.388	0.362	1.819	0.044		

Table 22 (cont.)

Stopped time (s)	GA (mg L^{-1})	Peak height						Slope	r^2
		1	2	3	$\overline{X}$	Blank- $\overline{X}$	SD		
40	0 (blank)	2.148	2.168	2.129	2.148	0.000	0.020	0.7506 ± 0.0328	0.9925
	0.25	2.010	2.091	2.051	2.051	0.097	0.041		
	0.50	1.767	1.726	1.767	1.753	0.395	0.023		
	1.0	1.320	1.320	1.320	1.320	0.828	0.000		
	1.5	1.036	0.996	0.996	1.009.	1.139	0.023		
	2.0	0.712	0.671	0.630	0.671	1.477	0.041		
	2.5	0.306	0.306	0.306	0.306	1.842	0.000		
50	0 (blank)	2.109	2.090	2.129	2.109	0.000	0.020	0.7880 ± 0.0106	0.9994
	0.25	1.906	1.906	1.906	1.906	0.208	0.000		
	0.50	1.687	1.687	1.687	1.687	0.427	0.000		
	1.0	1.250	1.294	1.250	1.265	0.849	0.025		
	1.5	0.857	0.900	0.944	0.900	1.214	0.044		
	2.0	0.507	0.551	0.507	0.522	1.592	0.025		
	2.5	0.113	0.113	0.113	0.113	2.001	0.000		
60	0 (blank)	2.188	2.129	2.129	2.148	0.000	0.034	0.7573 ± 0.0627	0.9950
	0.25	1.995	1.995	1.995	1.995	0.153	0.000		
	0.50	1.820	1.820	1.776	1.805	0.343	0.025		
	1.0	1.382	1.382	1.426	1.397	0.751	0.025		
	1.5	0.945	0.945	0.989	0.960	1.188	0.025		
	2.0	0.596	0.596	0.639	0.610	1.538	0.025		
	2.5	0.333	0.333	0.333	0.333	1.815	0.000		

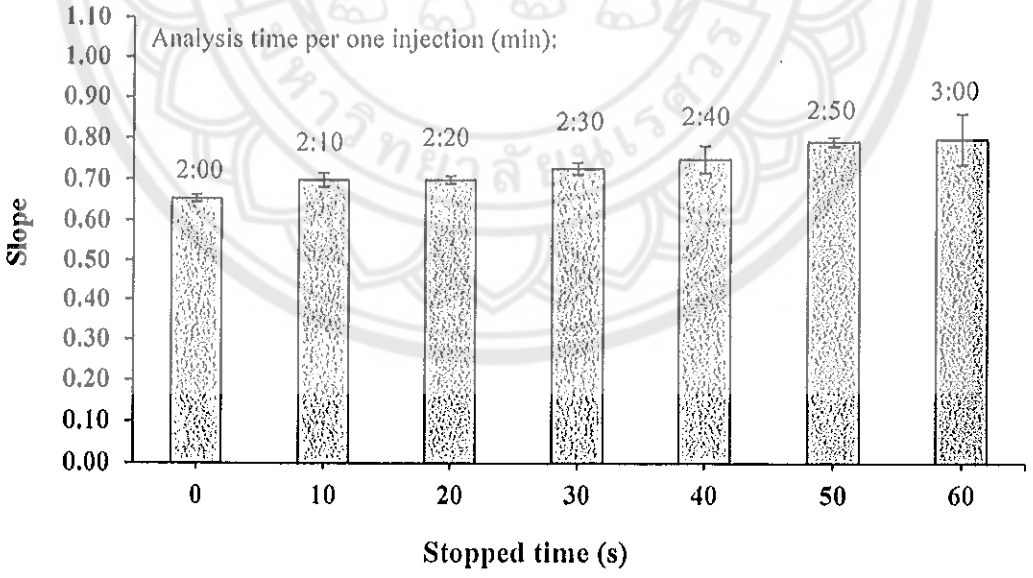


Figure 20 Effect of stopped times at reaction loop for ABTS assay on slope of GA (0.25 – 2.50 mg L^{-1})

2.1.5 Effect of reaction loop lengths

A reaction loop (RL) is placed for the merging zone of S + R_A before it was propelled by a carrier stream toward the detector. Volume of S + R_A zone depends on the length of reaction loop. A longer reaction loop length will increases the sensitivity (slope) of GA + $ABTS^{\bullet+}$ reaction but it increases an analysis time per one injection. To achieve high sensitivity and short analysis time, the effect of reaction loop lengths (20, 40, 60 and 80 cm) were optimized. A blank (water) and GA (0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 mg L^{-1}) were aspirated into the system using preliminary conditions in Table 18 and obtained conditions in 2.1.1 to 2.1.4. Results are shown in Table 23 and Figure 21. From the results, the longer length of reaction loop, the higher sensitivity was gained due to more volume of S and R_A. The analysis times increased when reaction loop lengths increased. Therefore, to compromise among the sensitivity, analysis time (2 min 50 sec) and precision requirement, a 40 cm of reaction loop length was chosen.

Table 23 Effect of reaction loop lengths on peak height and slope of GA standard solutions

Loop length (cm)	GA (mg L^{-1})	Peak height (mV)						Slope	r^2
		1	2	3	$\overline{X}$	Blank- $\overline{X}$	SD		
20	0 (blank)	2.129	2.109	2.129	2.122	0.000	0.011	0.6958 ± 0.0167	0.9964
	0.25	1.957	1.937	1.957	1.950	0.172	0.011		
	0.50	1.794	1.753	1.753	1.760	0.362	0.031		
	1.0	1.427	1.367	1.427	1.407	0.715	0.035		
	1.5	1.019	0.959	0.979	0.986	1.136	0.031		
	2.0	0.693	0.715	0.775	0.728	1.394	0.042		
	2.5	0.449	0.348	0.367	0.388	1.734	0.054		
40	0 (blank)	2.363	2.383	2.383	2.376	0.000	0.011	0.8679 ± 0.0232	0.9965
	0.25	2.149	2.128	2.128	2.135	0.241	0.012		
	0.50	1.946	1.965	1.965	1.959	0.417	0.011		
	1.0	1.498	1.559	1.559	1.538	0.838	0.035		
	1.5	1.091	1.111	1.152	1.118	1.258	0.031		
	2.0	0.725	0.704	0.704	0.711	1.665	0.012		
	2.5	0.115	0.134	0.216	0.155	2.221	0.054		
60	0 (blank)	2.617	2.500	2.539	2.552	0.000	0.060	0.9337 ± 0.0292	0.9957
	0.25	2.419	2.302	2.341	2.354	0.198	0.060		
	0.50	2.144	2.026	2.065	2.078	0.474	0.060		
	1.0	1.711	1.633	1.672	1.672	0.880	0.039		
	1.5	1.356	1.239	1.278	1.291	1.261	0.060		
	2.0	0.766	0.649	0.727	0.714	1.838	0.060		
	2.5	0.254	0.176	0.215	0.215	2.337	0.039		
80	0 (blank)	2.637	2.637	2.656	2.643	0.000	0.011	0.9930 ± 0.0266	0.9975
	0.25	2.422	2.422	2.441	2.428	0.215	0.011		
	0.50	2.145	2.145	2.204	2.165	0.478	0.034		
	1.0	1.750	1.750	1.769	1.756	0.887	0.011		

Table 23 (cont.)

Loop length (cm)	GA (mg L^{-1})	Peak height (mV)						Slope	r^2
		1	2	3	$\overline{X}$	Blank- $\overline{X}$	SD		
80	1.5	1.236	1.236	1.255	1.242	1.401	0.011		
	2.0	0.682	0.761	0.781	0.742	1.901	0.052		
	2.5	0.129	0.168	0.188	0.162	2.481	0.030		

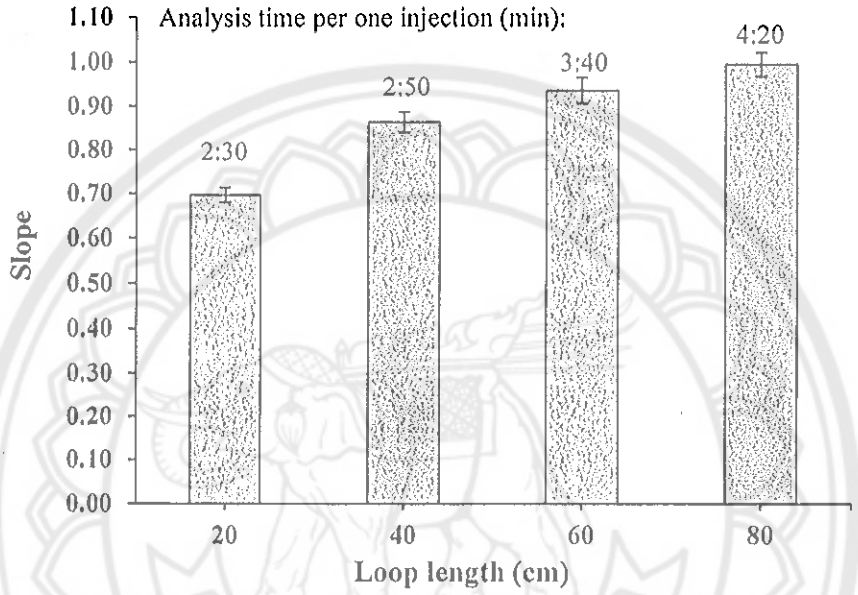


Figure 21 Effect of reaction loop lengths on slope of GA (0.25 – 2.50 mg L^{-1})

2.2 FC assay

2.2.1 Effect of FC concentrations

The FC concentration is a reagent to measure total phenolic compounds or any reducing substance in samples and, in this work, its concentration affects the sensitivity (slope), linearity and blank signal for the measurement. Thus, the FC concentrations were studied in the ratios of FC:water at 1:25, 1:20, 1:15, 1:10 and 1:5 v/v. A blank (water) and GA (5, 10, and 20 mg L^{-1}) were aspirated into the system using preliminary conditions in Table 18. Results are shown in Table 24 and Figure 22. It was found that the sensitivities were slightly increased with increasing FC concentrations from 1:25 to 1:15. The linearity (r^2) of all FC concentrations was more than 0.99 except at a ratio of 1:25. Moreover, high blank signals were found when FC

concentrations were increased, especially at ratios of 1:10 and 1:5. Therefore, the FC concentration at a ratio of 1:15 was selected for further studies, as it provided the highest sensitivity, good linearity and low lank signal.

Table 24 Effect of FC concentrations (ratios of FC:water) on peak height and slope of GA standard solutions

FC: water (v:v)	GA (mg L^{-1})	Peak height (mV)						Slope	r^2
		1	2	3	$\overline{X}$	$\overline{X}$ - blank	SD		
1:25	0 (blank)	0.059	0.059	0.059	0.059	0.000	0.000	0.1394 ± 0.0141	0.9899
	5	1.074	1.094	1.089	1.084	1.025	0.014		
	10	1.934	2.051	1.970	1.985	1.926	0.060		
	20	3.242	3.203	3.203	3.216	3.158	0.023		
1:20	0 (blank)	0.059	0.059	0.059	0.059	0.000	0.000	0.1423 ± 0.0114	0.9937
	5	1.016	0.996	0.996	1.001	0.947	0.014		
	10	1.895	1.875	1.875	1.882	1.823	0.011		
	20	3.184	3.164	3.174	3.174	3.115	0.014		
1:15	0 (blank)	0.059	0.059	0.059	0.059	0.000	0.000	0.1473 ± 0.0148	0.9900
	5	0.938	1.016	0.977	0.977	0.918	0.039		
	10	1.973	1.973	1.836	1.917	1.869	0.079		
	20	3.242	3.223	3.223	3.229	3.171	0.011		
1:10	0 (blank)	0.078	0.078	0.078	0.078	0.000	0.000	0.1292 ± 0.0121	0.9913
	5	0.918	0.898	0.879	0.898	0.820	0.020		
	10	1.719	1.719	1.719	1.719	1.641	0.000		
	20	2.891	2.852	2.871	2.871	2.793	0.020		
1:5	0 (blank)	0.098	0.117	0.098	0.104	0.000	0.000	0.1048 ± 0.0034	0.9990
	5	0.742	0.625	0.703	0.690	0.586	0.061		
	10	1.250	1.328	1.211	1.263	1.159	0.061		
	20	2.266	2.324	2.227	2.272	2.168	0.050		

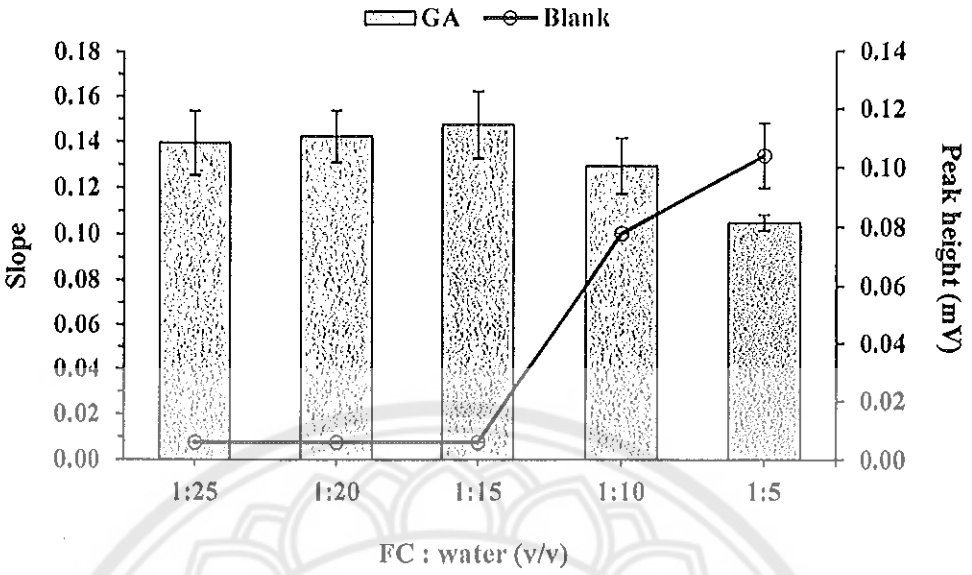


Figure 22 Effect of FC concentrations (ratios of FC:water) on slope of GA (5 – 20 mg L^{-1}) and peak height (mV) of blank signals

2.2.2 Effect of NaOH concentrations

A NaOH was used as an alkaline solution to give a blue color intensity of FC reagent and was affected the sensitivity (slope), linearity and blank signal for the determination. Thus, the effect of NaOH concentrations was examined at 0.15, 0.20, 0.25, 0.30 and 0.35 mol L^{-1}. A blank (water) and GA (2.5, 5.0, 10.0, 15.0 and 20.0 mg L^{-1}) were aspirated into the system using preliminary conditions in Table 18 and the obtained condition in 2.2.1. Results were indicated in Table 25 and Figure 23. The sensitivity increased when NaOH concentrations increased from 0.15 to 0.25 mol L^{-1}. Furthermore, the blank signals increased slightly with increasing NaOH concentrations at 0.25 to 0.35 mol L^{-1}. To achieve high sensitivity and good linearity (r^2 > 0.99), the NaOH concentration of 0.25 mol L^{-1} was chosen for further studies.

Table 25 Effect of NaOH concentrations on peak height and slope of GA standard solutions

NaOH (mol L^{-1})	GA (mg L^{-1})	Peak height (mV)						Slope	r^2
		1	2	3	$\overline{X}$	$\overline{X}$ - blank	SD		
0.15	0 (blank)	0.059	0.059	0.059	0.059	0.000	0.000	0.1184 ± 0.0049	0.9958
	2.5	0.343	0.343	0.343	0.343	0.284	0.000		
	5	0.694	0.694	0.694	0.694	0.635	0.000		
	10	1.396	1.361	1.361	1.373	1.314	0.020		
	15	1.853	1.888	1.868	1.869	1.811	0.012		
	20	2.415	2.450	2.430	2.432	2.373	0.018		
0.20	0 (blank)	0.059	0.059	0.059	0.059	0.000	0.000	0.1293 ± 0.0057	0.9969
	2.5	0.410	0.375	0.393	0.393	0.334	0.018		
	5	0.727	0.762	0.762	0.750	0.692	0.020		
	10	1.395	1.430	1.414	1.413	1.354	0.018		
	15	2.099	2.134	2.160	2.131	2.072	0.036		
	20	2.626	2.626	2.626	2.626	2.568	0.000		
0.25	0 (blank)	0.078	0.078	0.078	0.078	0.000	0.000	0.1433 ± 0.0065	0.9935
	2.5	0.400	0.400	0.400	0.400	0.322	0.000		
	5	0.855	0.855	0.969	0.893	0.815	0.066		
	10	1.690	1.690	1.690	1.690	1.612	0.066		
	15	2.260	2.298	2.279	2.279	2.200	0.019		
	20	2.943	2.981	2.943	2.955	2.877	0.029		
0.30	0 (blank)	0.078	0.078	0.078	0.078	0.000	0.000	0.1372 ± 0.0091	0.9927
	2.5	0.384	0.384	0.384	0.384	0.306	0.000		
	5	0.834	0.834	0.834	0.834	0.759	0.000		
	10	1.637	1.602	1.602	1.614	1.536	0.020		
	15	2.229	2.229	2.229	2.229	2.151	0.020		
	20	2.786	2.820	2.786	2.797	2.719	0.020		
0.35	0 (blank)	0.078	0.078	0.078	0.078	0.000	0.000	0.1320 ± 0.0098	0.9917
	2.5	0.366	0.366	0.366	0.366	0.288	0.000		
	5	0.819	0.819	0.819	0.819	0.741	0.000		
	10	1.564	1.564	1.564	1.564	1.486	0.000		
	15	2.146	2.146	2.146	2.146	2.068	0.000		
	20	2.696	2.696	2.696	2.696	2.618	0.000		

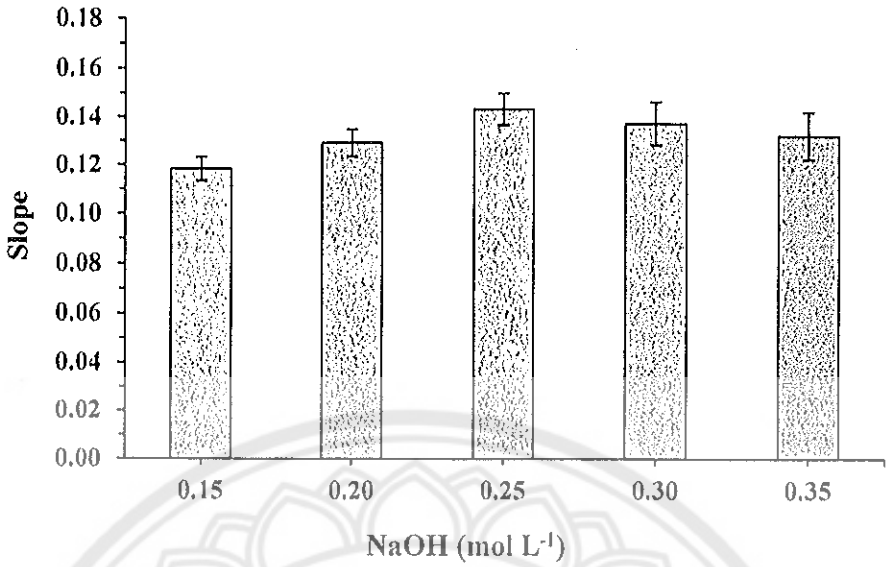


Figure 23 Effect of NaOH concentrations on slope of GA (2.5 – 20 mg L^{-1})

2.2.3 Effect of flow rates

From a reasonable similarity as a continuous flow system using ABTS assay, the effect of flow rates of the system using FC assay was also studied at 1.6 and 2.0 mL min^{-1}. Using preliminary conditions in Table 18 and the obtained conditions in 2.2.1 and 2.2.2, a blank (water) and GA (2.5, 5.0, 10.0, 15.0 and 20.0 mg L^{-1}) were aspirated into the system. Table 26 and Figure 24 showed that the highest sensitivity was found at flow rate of 1.6 mL min^{-1}, but it gave long analysis time (4:40 min). Both flow rates gave the symmetric peak. Thus, the flow rate of 1.6 mL min^{-1} was selected as it provided higher sensitivity, good symmetric peak and acceptable analysis time (4 min 40 sec).

Table 26 Effect of flow rates on peak height and slope of GA standard solutions

Flow rate (mL min^{-1})	GA (mg L^{-1})	Peak height (mV)						Slope	r^2
		1	2	3	$\overline{X}$	$\overline{X}$ - blank	SD		
1.6	0 (blank)	0.059	0.059	0.059	0.059	0.000	0.000	0.1403 ± 0.0061	0.9923
	2.5	0.410	0.410	0.410	0.410	0.352	0.000		
	5	0.859	0.859	0.840	0.853	0.794	0.011		
	10	1.641	1.621	1.660	1.641	1.582	0.020		
	15	2.321	2.324	2.324	2.324	2.266	0.000		
	20	2.852	2.852	2.852	2.852	2.793	0.000		
2.0	0 (blank)	0.059	0.059	0.059	0.059	0.000	0.000	0.1363 ± 0.0064	0.9933
	2.5	0.410	0.391	0.410	0.400	0.342	0.011		
	5	0.824	0.859	0.859	0.848	0.789	0.021		
	10	1.602	1.621	1.621	1.612	1.556	0.011		
	15	2.246	2.207	2.227	2.227	2.168	0.020		
	20	2.813	2.793	2.793	2.800	2.741	0.011		

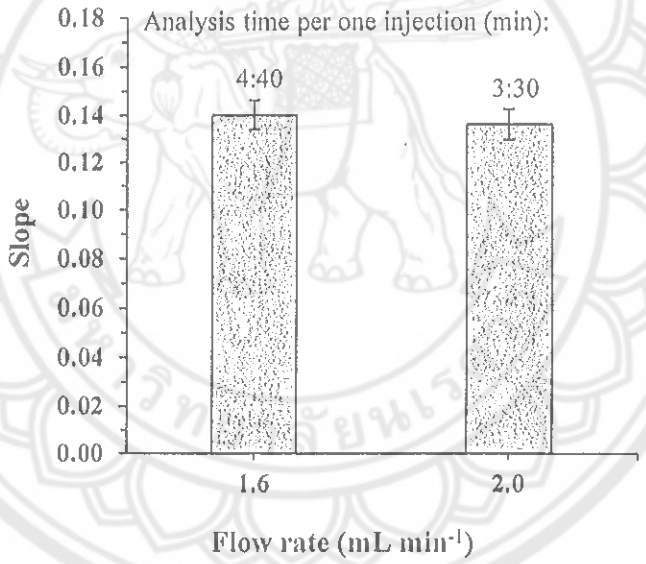


Figure 24 Effect of flow rates of the system on slope of GA (2.5 – 20 mg L^{-1})

2.2.4 Effect of reaction loop lengths and aspiration time ratios of S, FC and NaOH solutions

The reaction loop lengths (volume of GA (S) + FC (R_F) + NaOH (R_N)) and aspiration times of S, R_F and R_N (related to volume of S, R_F and R_N as expressing second) affect to the sensitivity of the reaction. To maximize sensitivity and

to obtain short analysis time, the effect of reaction loop lengths (20, 30, 40 and 50 cm) and aspiration time ratios of S, R_F and R_N (1:1:1, 2:1:1, 3:1:1 and 4:1:1, s:s:s) were studied. Under preliminary conditions in Table 18 and obtained conditions in 2.2.1 to 2.2.3, a blank (water) and GA (20.0 mg L^{-1}) were aspirated into the system. Results are shown in Table 27 and Figure 25. The sensitivity increased slightly when reaction loop lengths increased. For all aspiration time ratios, the increasing aspiration times of standard/sample (S) volumes showed the rising peak height. Moreover, an analysis time increased when both reaction loop lengths and aspirations time of S in the ratio of S:FC:NaOH increased. Thus, the reaction loop lengths of 20 cm and aspiration time ratio of 2:1:1 (s:s:s) of $S:R_F:R_N$ were chosen to obtain an enough peak height, acceptable precision and adequate analysis time (3 min 50 sec).

Table 27 Effect of reaction loop lengths (cm) and aspiration time ratios (s:s:s) of standard (S), FC (R_F) and NaOH (R_N) solutions on peak height of GA standard solutions

Loop length (cm)	$S:R_F:R_N$ (s:s:s)	GA (mg L^{-1})	Peak height (mV)					
			1	2	3	$\overline{X}$	$\overline{X}$ - blank	SD
20	1:1:1	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
		20	2.227	2.344	0.285	0.285	2.246	0.083
	2:1:1	0 (blank)	0.020	0.020	0.020	0.020	0.000	0.000
		20	3.047	3.006	3.066	3.040	3.020	0.031
	3:1:1	0 (blank)	0.020	0.020	0.020	0.020	0.000	0.000
		20	3.356	3.340	3.320	3.339	3.319	0.018
	4:1:1	0 (blank)	0.020	0.020	0.020	0.020	0.000	0.000
		20	3.320	3.301	3.320	3.314	3.294	0.011
30	1:1:1	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
		20	2.341	2.306	2.285	2.311	2.271	0.028
	2:1:1	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
		20	3.105	3.086	3.125	3.106	3.066	0.020
	3:1:1	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
		20	3.455	3.730	3.457	3.547	3.508	0.159
	4:1:1	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
		20	3.418	3.418	3.457	3.431	3.392	0.023
40	1:1:1	0 (blank)	0.059	0.053	0.056	0.056	0.000	0.000
		20	2.461	2.441	2.422	2.441	2.386	0.020
	2:1:1	0 (blank)	0.052	0.059	0.059	0.056	0.000	0.000
		20	3.223	3.301	3.261	3.262	3.205	0.039
	3:1:1	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
		20	3.574	3.555	3.535	3.555	3.516	0.020
	4:1:1	0 (blank)	0.020	0.020	0.020	0.020	0.000	0.000
		20	3.555	3.535	3.555	3.548	3.529	0.011

Table 27 (cont.)

Loop length (cm)	S:R_F:R_N (s:s:s)	GA (mg L^{-1})	Peak height (mV) 1	2	3	$\overline{X}$	$\overline{X}$ - blank	SD
50	1:1:1	0 (blank)	0.059	0.059	0.059	0.059	0.000	0.000
		20	2.441	2.422	2.402	2.422	2.363	0.020
	2:1:1	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
		20	3.262	3.281	3.301	3.281	3.212	0.020
	3:1:1	0 (blank)	0.020	0.020	0.020	0.020	0.000	0.000
		20	3.633	3.574	3.594	3.600	3.581	0.030
	4:1:1	0 (blank)	0.020	0.020	0.020	0.020	0.000	0.000
		20	3.633	3.652	3.672	3.652	3.633	0.020

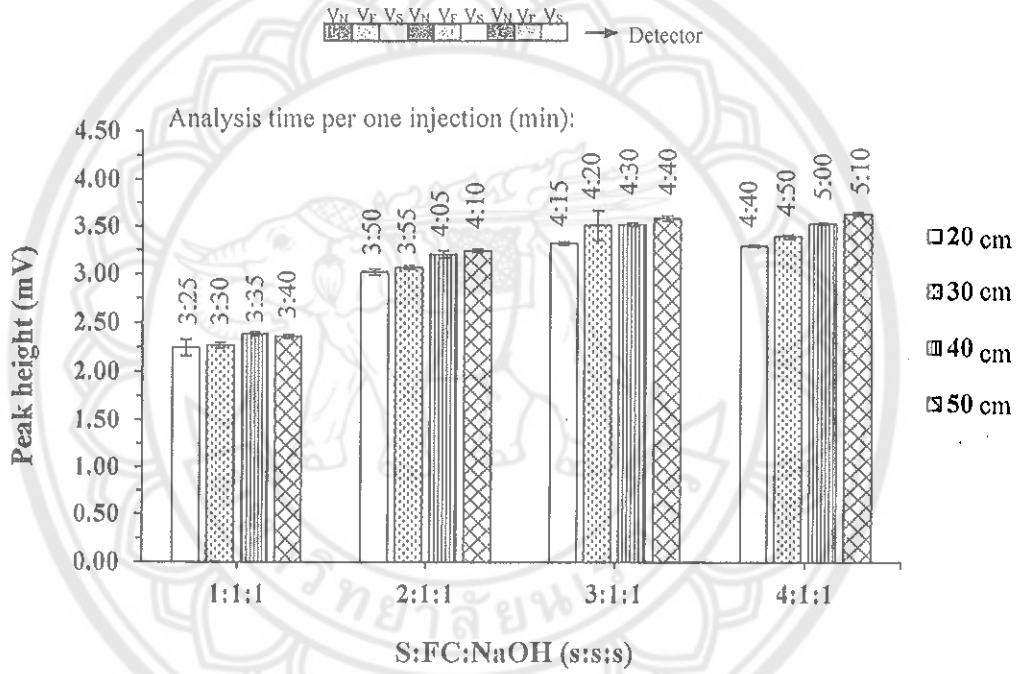


Figure 25 Effect of reaction loop lengths (cm) and aspiration times (s:s:s) of standard/sample (S), FC (R_F) and NaOH (R_N) solutions on peak height of GA (20 mg L^{-1}) and analysis time (min) per one injection

2.2.5 Effect of stopped times at reaction loop

The effect of stopped time was studied in order to increase sensitivity of GA+FC+NaOH reaction. Various stopped times of 0, 30, 60, 120 and 300 s were varied and optimized. Using preliminary conditions in Table 18 and obtained conditions in 2.2.1 to 2.2.4, a blank (water) and GA (20.0 mg L^{-1}) were aspirated into the system. Results are shown in Table 28 and Figure 26. Peak heights of GA and

analysis time per one injection increased when stopped times increased. To provide an acetable peak height and short analysis time (3 min 30 sec), a nonstop time of GA + FC + NaOH reaction was chosen for further studies.

Table 28 Effect of stopped times at reaction loop on peak height of GA standard solutions

Stopped time (s)	GA (mg L^{-1})	Peak height (mV)					
		1	2	3	$\overline{X}$	$\overline{X}$ - blank	SD
0	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
	20	3.164	3.164	3.164	3.164	3.125	0.000
30	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
	20	3.262	3.281	3.281	3.275	3.236	0.011
60	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
	20	3.418	3.320	3.340	3.359	3.320	0.052
120	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
	20	3.379	3.412	3.388	3.398	3.359	0.028
300	0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000
	20	3.457	3.466	3.476	3.466	3.427	0.009

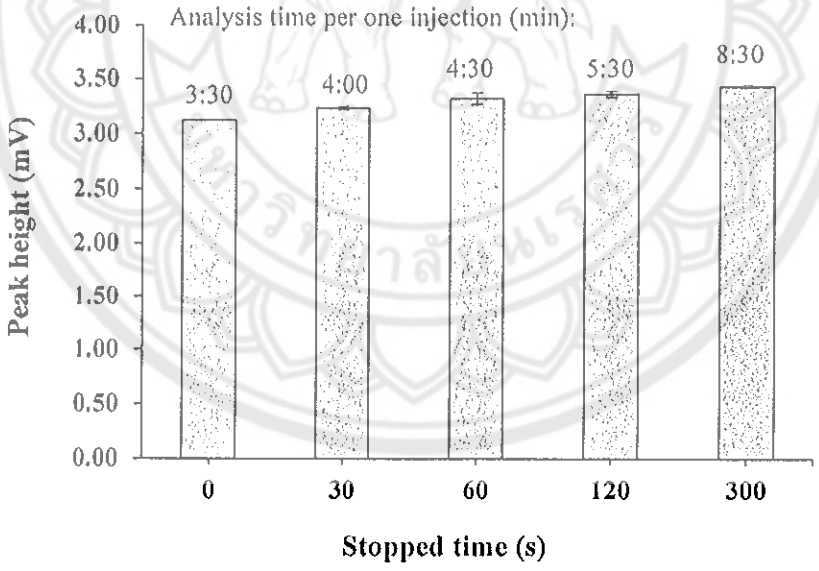


Figure 26 Effect of stopped times at reaction loop for FC assay on peak height of GA (20 mL^{-1}) and analysis time (min) per one injection

2.3 Interference study for ABTS and FC assays

There are many chemical compositions in herbs such as phenolic compounds, vitamins, minerals, sugars, organic acids, and inorganic acids. Some chemical species could possibly interfered the reactions of antioxidant compounds with $ABTS^{\bullet+}$ and FC reagent solutions. Two continuous flow systems using ABTS and FC assays were operated under the selected conditions as described above (2.1.1 to 2.1.5. and 2.2.1 to 2.2.5). A 100 mg L^{-1} of glucose (Ajax Finechem), fructose (Merck), citric acid (BDH), succinic acid (Fluka), sodium chloride (Meck) and sodium sulfate (Fisher Scientific) were used as interference compounds and investigated for the systems. The results were shown in Table 29. It was found that no detector signals were found for all interference compounds for both systems of ABTS and FC assays. Thus, it could be concluded that all typical interference compounds was not reacted with both $ABTS^{\bullet+}$ and FC reagents.

Table 29 Effect of interferences on peak height of $ABTS^{\bullet+}$ and FC reagent solutions

Interference Compound	Concentration added (mg L^{-1})	Peak height (mV)					
		ABTS assay			FC assay		
		$\bar{X}$ (n=3)	Blank - $\bar{X}$	%RSD	$\bar{X}$ (n=3)	Blank - $\bar{X}$	%RSD
Blank (water)	0	1.660			0.020		
Succinic acid	100	1.660	0.000	0	0.020	0.000	0
Citric acid	100	1.660	0.000	0	0.020	0.000	0
Glucose	100	1.660	0.000	0	0.020	0.000	0
Frutose	100	1.660	0.000	0	0.020	0.000	0
Cl^-	100	1.660	0.000	0	0.020	0.000	0
SO_4^{2-}	100	1.660	0.000	0	0.020	0.000	0

2.4 Summary of conditions used of the continuous flow systems using ABTS and FC assays for the determination of antioxidant capacity and total phenolic compounds, respectively

The continuous flow systems using ABTS and FC assays for the determination of antioxidant capacity and total phenolic compounds, respectively, are presented in Figure 7 and the optimum conditions are summarized in Table 30.

Table 30 Conditions used of the continuous flow systems using ABTS and FC assays for the determination of antioxidant capacity and total phenolic compounds, respectively

Parameters	Conditions used	
	ABTS assay	FC assay
Continuous flow system:		
Carrier solution (C_s)	Water	Water
Standard/sample (S)	Gallic acid	Gallic acid
Reagent solutions: R_A	$ABTS^{\bullet+}$ in water (expressed as 0.84 absorbance)	-
R_F	-	FC:water (1:15)
R_N		NaOH (0.25 mol L^{-1})
Flow rate	1.6 mL min^{-1}	1.6 mL min^{-1}
Reaction loop (RL) length	40 cm	20 cm
Volumes of S:R_A and S:R_F:R_N	27 µL : 27 µL (equal to 1s and 1 s of aspiration times)	54 µL: 27 µL: 27 µL (equal to 2s:1s:1 s of aspiration times)
Stopped time at RL	30 s	0 s
Detection wavelength	730 nm	728 nm
Operation times of the system:		
1) Filling steps of:		
- C_s to RL, FTC and W	120 s	130 s
- S to RL, WC and W	65 s	40 s
- R_A to RL, WC and W	65 s	-
- R_F to RL, WC and W	-	45 s
- R_N to RL, WC and W	-	42 s
- C_s to RL, WC and W	160 s	120 s

Table 30 (cont.)

Parameters	Conditions used	
	ABTS assay	FC assay
Operation times of the system:		
2) Loading steps of:		
- Rapid sequenced aspiration of S, and R_A at RL to WC and W	20 s (S:R_A = 1s:1s)	-
- Rapid sequenced aspiration of S, R_F and R_N at RL to WC and W	-	20 s (S:R_F:R_N = 2s:1s:1s)
3) Injection and cleaning steps of:		
- S + R_A zone at RL to FC and W	250 s	-
- S + R_F + R_N zone at RL to FC and W	-	190 s
4) Cleaning steps of:		
- C_s to RL, FTC and W	250 s	250 s
- S to RL, WC and W	80 s	90 s
- R_A to RL, WC and W	80 s	-
- R_F to RL, WC and W	-	90 s
- R_N to RL, WC and W	-	90 s
- C_s to RL, WC and W	160 s	150 s

2.5 Analytical performance characteristics of the continuous flow systems using ABTS and FC assays for the determination of antioxidant capacity and total phenolic compounds, respectively

Under the continuous flow systems used in Figure 7 and optimum conditions used in Table 30, blank (water) 0.25 - 2.5 mg L^{-1} of gallic acid standard solutions for ABTS assay and 2.5 – 15.0 mg L^{-1} of GA standard solutions for FC assay were analysed to validate the two proposed systems. For the system of ABTS assay, all calibration data are shown in Table 31 and Figure 27. Linear range of GA was 0.25 – 2.50 mg L^{-1} ($y = 0.7836x - 0.0653$, $r^2 = 0.9992$). Limit of detection (LOD) and limit of quantification (LOQ) were 0.03 and 0.10 mg L^{-1}, respectively. The RSD was 0.05 to 8.3 % and the sample throughput was 21 injections per hour. For the system of FC assay, the results are exhibited in Table 32 and Figure 28. Linear range was obtained in the range 2.5 – 15.0 mg L^{-1} of GA ($y = 0.1772x + 0.1377$, $r^2 = 0.9933$). LOD and LOQ were 0.04 and

0.15 mg L^{-1}, respectively. The RSD was of 0.01 to 5.3 % and the sample throughput was 17 injections per hour.

Table 31 Calibration data for the determination of antioxidant capacity using ABTS assay

GA	Peak height (mV)						
(mg L^{-1})	1	2	3	$\bar{X}$	Blank - $\bar{X}$	SD	%RSD
0 (blank)	2.402	2.402	2.402	2.402	0.000	0.000	0.0
0.25	2.246	2.266	2.227	2.246	0.156	0.020	0.9
0.50	2.090	2.129	2.090	2.103	0.299	0.023	1.1
1.0	1.680	1.680	1.699	1.686	0.716	0.011	0.7
1.5	1.309	1.328	1.270	1.302	1.100	0.036	2.3
2.0	0.859	0.879	0.905	0.881	1.521	0.023	2.6
2.5	0.508	0.527	0.508	0.514	1.888	0.023	2.2

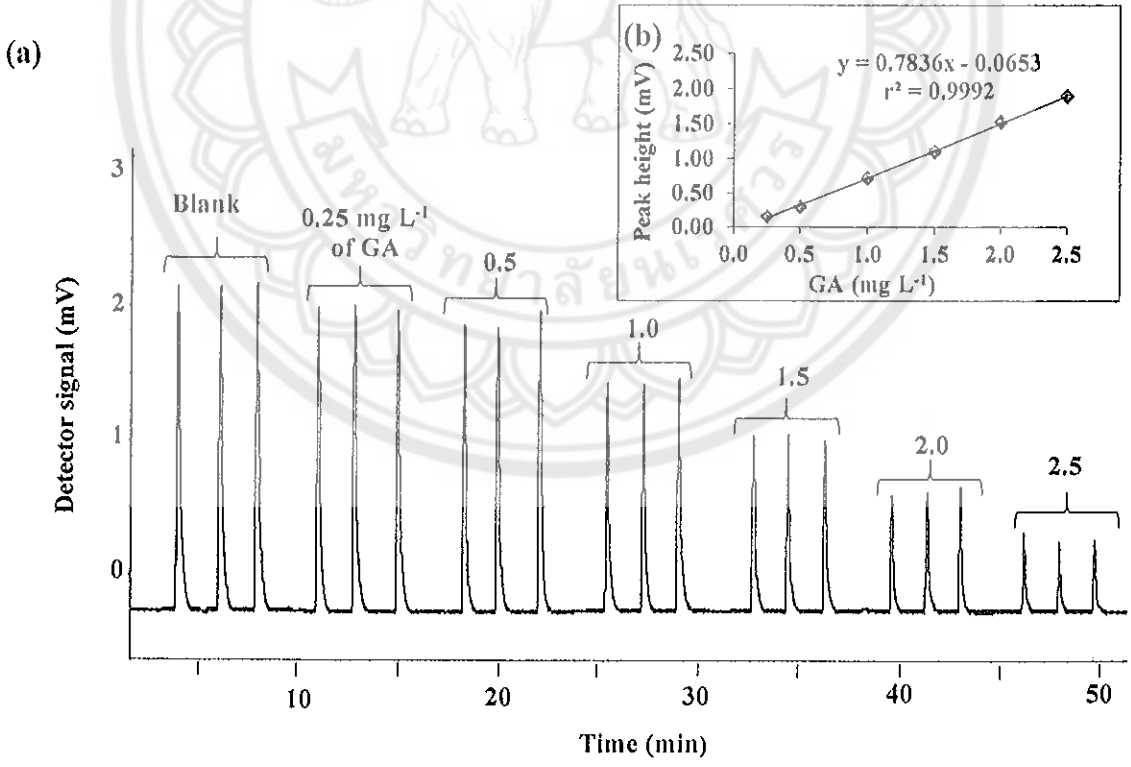


Figure 27 (a) Typical of detector siganals and (b) calibration graph of GA for the determination of antioxidant capacity using ABTS assay

Table 32 Calibration data for the determination of total phenolic compounds using FC assay

GA (mg L^{-1})	Peak height (mV)						
	1	2	3	$\overline{X}$	Blank - $\overline{X}$	SD	%RSD
0 (blank)	0.039	0.039	0.039	0.039	0.000	0.000	0.0
2.5	0.547	0.547	0.547	0.547	0.508	0.000	0.0
5.0	1.074	1.055	1.055	1.061	1.022	0.011	1.1
7.5	1.582	1.582	1.563	14.576	1.537	0.011	0.7
10.0	2.012	2.012	2.032	2.018	1.979	0.011	0.6
12.5	2.402	2.402	2.441	2.415	2.976	0.023	0.9
15.0	2.754	2.734	2.754	2.747	2.708	0.011	0.4

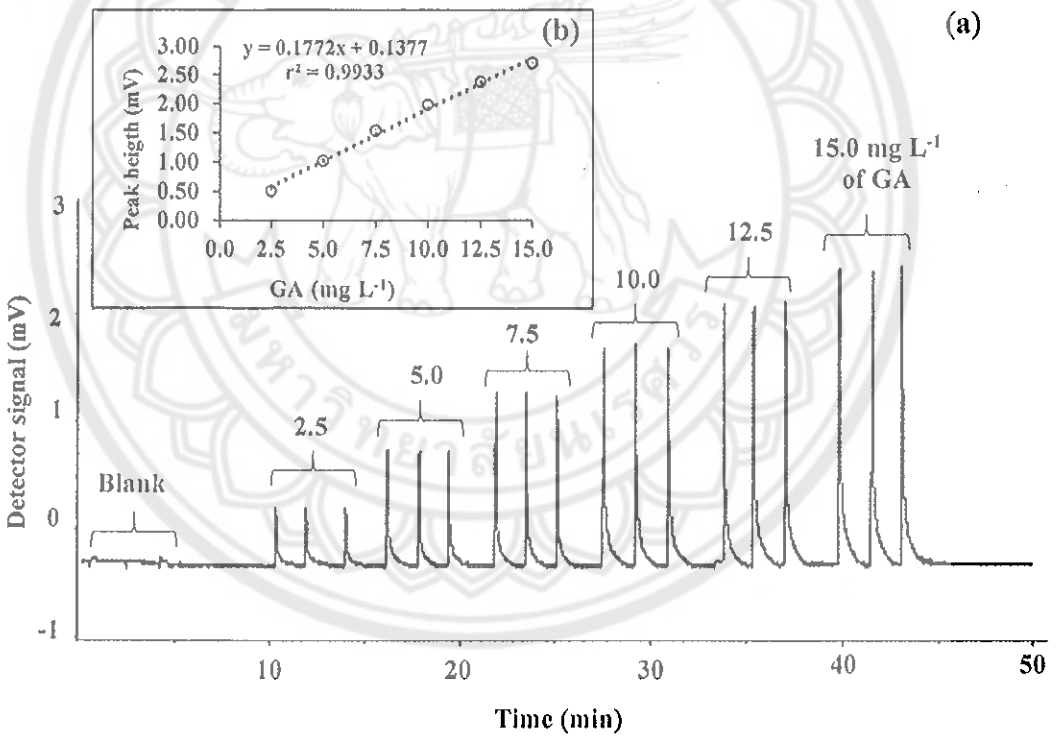


Figure 28 (a) Typical of detector signals and (b) calibration graph of GA for the determination of total phenolic compounds using FC assay

3. Application of the continuous flow systems using ABTS and FC assays to real samples of teas and herbal teas

Under optimum conditions used, the twenty sample of teas and Thai herbal teas were determined contents of antioxidant capacity and total phenolic compounds by the continuous flow spectrophotometer systems using ABTS and FC assay, respectively. These antioxidant capacity and total phenolic compound contents were reported as gallic acid equivalent. Results are summarized in Table 33. Results obtained in Table 33 of the two proposed systems were validated with other spectrophotometric method by microplate reader. For ABTS assay, the contents of antioxidant activities in samples were found in the range of 56 ± 1 – 5952 ± 202 mg GA kg^{-1} of dry weight. Makhaam Pom tea was found the highest antioxidant capacity contents, while Mara Khee Nok was the lowest contents. Moreover, the antioxidant capacity contents obtained from the proposed system using ABTS assay were in good agreement with those obtained from microplate reader using ABTS assay (t-test of $t_{calculation}$ = 0.14 and $t_{critical}$ = 2.10, 95% confidence level). For FC assay, the contents of total phenolic compounds in samples were found in the range of 216 ± 4 – 9035 ± 287 mg GA kg^{-1} of dry weight. Cha Keqw Yepun was found the highest contents of total phenolic compounds, while Mara Khee Nok was the lowest contents. By validation the proposed method using FC assay with the microplate reader method using FC assay, the contents of total phenolic compounds from both methods were obtained in good agreement of results (t-test of $t_{calculation}$ = 0.38 and $t_{critical}$ = 2.10, 95% confidences level).

Table 33 Contents of antioxidant capacity (GA equivalent; mg L^{-1} and mg kg^{-1}) and total phenolic compounds (GA equivalent; mg L^{-1} and mg kg^{-1}) in tea and herbal tea samples, as determined by the proposed systems using ABTS and FC assays

No	Tea and herbal tea	Concentration found and % relative standard deviation (n=3)											
		ABTS assay						FC assay					
		Continuous flow system			Microplate reader			Continuous flow system			Microplate reader		
		mg L^{-1} [a]	mg kg^{-1}	%RSD	mg L^{-1}	mg kg^{-1}	%RSD	mg L^{-1}	mg kg^{-1}	%RSD	mg L^{-1}	mg kg^{-1}	%RSD
1	Yaa Nuat Maeo	1.2±0.01[b] (119±0.01)[c]	1193±1[d]	0.8	0.2±0.03 (116±0.03)	1161± 82	15.0	13.1±0.1[b] (263±0.1)[c]	2628±13[d]	0.8	0.4±0.01 (176±0.01)	1759±44	2.5
2	Makhaam Khaek	1.8±0.01 (89±0.01)	884±7	0.5	0.2±0.01 (90±0.01)	902±50	5.0	4.5±0.1 (225±0.1)	2253±23	2.2	0.6±0.02 (304±0.02)	3041±88	3.3
3	Matuum	2.1±0.02 (53±0.02)	531±6	1.0	0.1±0.01 (70±0.01)	699±1	10.0	10.8±0.2 (270±0.2)	2697±40	1.9	1.0±0.04 (506±0.04)	5060±220	4.0
4	Makhaam Pom	1.2±0.1 (597±0.1)	5952± 202	8.3	0.2±0.01 (590±0.01)	5883± 229	5.0	14.2±0.1 (711±0.1)	7090±32	0.7	0.3±0.01 (718±0.01)	7168±219	3.3
5	Chumhet Thet	0.5±0.01 (24±0.01)	237±7	2.0	0.03±0.004 (65±0.004)	649±96	13.3	5.9±0.001 (98±0.001)	972±1	0.02	0.5±0.06 (133±0.06)	1281±197	12.1
6	Khing	2.2±0.03 (22±0.03)	215±3	1.4	0.04±0.01 (19±0.01)	186±69	37.3	3.2±0.1 (53±0.1)	525±11	3.1	0.1±0.001 (45±0.001)	453±1	1.0
7	Bua Bok	2.3±0.01 (23±0.01)	230±1	0.4	0.3±0.02 (21±0.02)	208±12	6.7	5.7±0.3 (71±0.3)	710±32)	5.3	0.5±0.02 (81±0.02)	804±29	4.0
8	Thaowan Prieng	1.7±0.02 (17±0.02)	172±2	1.2	0.03±0.007 (18±0.007)	175±33	23.3	7.1±0.1 (118±0.1)	1178±11	1.4	0.4±0.07 (97±0.07)	966±177	17.5
9	Khamin Khruea	0.4±0.01 (7±0.01)	71±2	2.5	0.1±0.002 (6±0.002)	63±2	2.0	4.5±0.1 (45±0.1)	445±12	2.2	0.4±0.02 (43±0.02)	428±18	5.0

Table 33 (cont.)

No	Tea and herbal tea	Concentration found and % relative standard deviation (n=3)											
		ABTS assay						FC assay					
		Continuous flow system			Microplate reader			Continuous flow system			Microplate reader		
		mg L^{-1} [a]	mg kg^{-1}	%RSD	mg L^{-1}	mg kg^{-1}	%RSD	mg L^{-1}	mg kg^{-1}	%RSD	mg L^{-1}	mg kg^{-1}	%RSD
10	Kha Chay Dum	0.6±0.01 (10±0.01)	101±1	1.7	0.01±0.002 (5±0.002)	47±10	20.0	4.5±0.1 (45±0.1)	447±12	2.2	0.4±0.05 (69±0.05)	730±15	12.5
11	Mon	2.1±0.04 (26±0.04)	261±5	1.9	0.1±0.01 (24±0.01)	224±35	10.0	6.2±0.2 (155±0.2)	1549±42	3.2	0.3±0.01 (162±0.01)	1621±44	3.3
12	Krachip Daeng	1.6±0.01 (81±0.01)	805±7	0.6	0.2±0.002 (76±0.002)	764±10	1.0	3.2±0.1 (159±0.1)	1594±31	3.1	0.4±0.01 (193±0.01)	1925±44	10.0
13	Chaa Phluu	1.8±0.1 (29±0.1)	291±2	5.5	0.1±0.01 (24±0.01)	236±24	10.0	6.33±0.1 (106±0.1)	1056±10	1.6	0.23±0.01 (115±0.01)	3041±88	4.3
14	Cha Em Ted	0.3±0.01 (17±0.01)	172±7	3.3	0.2±0.01 (19±0.01)	188±7	5.0	5.3±0.2 (88±0.2)	877±28	3.8	0.3±0.01 (136±0.01)	1355±44	3.3
15	Takhrai	0.6±0.01 (9±0.01)	92±2	1.7	0.17±0.01 (11±0.01)	105±8	5.9	7.1±0.001 (35±0.001)	353±1	0.01	0.7±0.01 (44±0.01)	437±5	1.4
16	Yaa Puk King	1.8±0.001 (9±0.001)	87±1	0.05	0.2±0.01 (10±0.01)	97±4	5.0	5.6±0.2 (28±0.2)	279±11	3.6	0.07±0.001 (33±0.001)	328±1	1.4
17	Mara Khee Nok	0.4±0.01 (6±0.01)	56±1	2.5	0.2±0.002 (7±0.002)	65±1	1.0	3.5±0.1 (22±0.1)	216±4	2.9	0.5±0.04 (29±0.04)	291±22	8.0
18	Kheelek	1.7±0.01 (86±0.01)	858±7	0.6	0.2±0.01 (75±0.01)	750±12	5.0	4.9±0.001 (244±0.001)	2444±1	0.02	0.52±0.01 (258±0.01)	2576±44	1.9
19	Cha keaw	2.3±0.01 (578±0.01)	5774±36	0.4	0.1±0.005 (569±0.005)	5692± 236	5.0	3.3±0.001 (815±0.001)	8151±1	0.03	6.3±0.1 (791±0.1)	7911±99	1.6

Table 33 (cont.)

No	Tea and herbal tea	Concentration found and % relative standard deviation (n=3)											
		ABTS assay						FC assay					
		Continuous flow system			Microplate reader			Continuous flow system			Microplate reader		
		mg L^{-1} [a]	mg kg^{-1}	%RSD	mg L^{-1}	mg kg^{-1}	%RSD	mg L^{-1}	mg kg^{-1}	%RSD	mg L^{-1}	mg kg^{-1}	%RSD
20	Cha Keqw Yepun	1.8±0.01 (450±0.01)	4499±14	0.6	0.09±0.002 (444±0.002)	4440± 118	2.2	7.2±0.2 (904±0.2)	9035±287	3.8	5.3±0.04 (667±0.04)	4447±50	0.8

[a] Mean ± standard deviation (n = 3), [b] Antioxidant capacity contents (using ABTS assay) and total phenolic compounds (using FC assay) were calculated by comparing the gallic acid calibration graph, [c] Actual antioxidant capacity contents (using ABTS assay) and total phenolic compounds (using FC assay) were obtained by calculating dilution factor of each sample, [d] Actual antioxidant capacity contents (using ABTS assay) and total phenolic compounds (using FC assay) were obtained by calculating milligram per kilogram of each dried sample

CHAPTER V

CONCLUSIONS

In this work, three systems were studied for antioxidant analysis. There are 1) the HPLC system with DAD detector and 2) two continuous flow systems with UV/Vis spectrophotometer.

For the HPLC system, it was developed, optimized and validated for the simultaneous determination of gallic acid (GA), gallocatechin (GC), catechin (C), epicatechin (EC), and epigallocatechin gallate (EGCG) in dried-crude extract of banana waste (e.g. raw peel, ripe peel, raw hand stalk, ripe hand stalk, raw bunch stalk and ripe bunch stalk) and chromatographic fingerprint analysis. Under optimum conditions, the dried-crude extracts were prepared using 90% v/v methanol, determined under isocratic elution using a C18 analytical column and a mobile phase of acetonitrile and 0.1% v/v formic acid (15:85 v/v) and detected at 275 nm. The order of elution was GA, GC, C, EC, and EGCG, with retention times of 3.86 ± 0.06, 4.65 ± 0.07, 7.95 ± 0.12, 11.19 ± 0.14 and 13.24 ± 0.17 min, respectively. The analysis time per chromatogram of a mixed standard solution and a sample solution were 20 and 50 min, respectively. The linear calibration graphs were in the range 0.25 - 20 mg L^{-1} of GA and 0.5 – 30 mg L^{-1} of GC, C, EC, and EGCG, respectively. Limit of detections (LOD) were 0.01, 0.07, 0.10, 0.01 and 0.02 mg L^{-1} of GA, GC, C, EC, and EGCG, respectively. Relative standard deviations (RSD) and recoveries were obtained in the ranges of 0.2 - 11.1 % and 59 ± 1 – 128 ± 1 %, respectively. The propose HPLC system was successfully applied to real sample of banana crude extracts. From the results, the proposed HPLC system could be determined four antioxidant compounds for sample analyses because of interferences found in all sample. Contents of GA, C and EC could be found in all parts of banana crude extracts. Contents of EGCG were found in raw peel, ripe peel, raw hand stalk and raw bunch stalk extracts. The highest contents of GA, C and EGCG were found in raw bunch stalk extract and the highest contents of EC were found in ripe bunch stalk extract. For the chromatographic fingerprint analysis, the chromatographic fingerprint patterns of peel, hand stalk and bunch stalk extracts were successfully established. Six marker

peaks were obtained in peel, hand stalk and bunch stalk of banana crude extracts. For peel and hand stalk crude extracts, retention times (RT) of six marker peaks were at 4.67 ± 0.17 min (peak 1), 5.85 ± 0.21 min (peak 2), 10.03 ± 0.18 min (peak 3), 11.30 ± 0.06 min (peak 4, EC), and 14.50 ± 0.13 min (peak 5) and 17.55 ± 0.14 min (peak 6). In bunch stalk crude extracts, the RTs of six marker peaks were 4.67 ± 0.17 min (peak 1), 5.85 ± 0.21 min (peak 2), 10.03 ± 0.18 min (peak 3), 11.30 ± 0.06 min (peak 4, EC), 14.50 ± 0.13 min (peak 5) and 22.83 ± 0.06 min (peak 6). Precisions (RSD) of relative retention times (RRT) of all marker peaks were in the range of 0.5 – 4.5 %. The proposed HPLC method was also performed simple and rapid extraction, good accuracy and precision. It could be said that banana waste extracts could be an alternative sources of antioxidant compounds of GA, C, EC and EGCG for the utilization in other applications such as agricultural feed stuffs (e.g. pigs, cattle and other animals), and additives in cosmetics and medicines.

For two continuous flow systems with UV/Vis spectrophotometer were developed, optimized and validated for the determination antioxidant capacity using ABTS assay and total phenolic compounds using FC assay in tea and herbal tea samples. For the continuous flow system using ABTS assay, the optimum conditions of a 0.84 A of $ABTS^{\bullet+}$ concentration, a acetate buffer (0.02 mol L^{-1}, pH 4.5) of carrier solution, a 1.6 mL min^{-1} of flow rate, a 30 sec of stopped time at reaction loop (RL), a 40 cm of RL length, and a 730 nm of detection wavelength, were selected. A linear calibration graph was 0.25 – 2.5 mg L^{-1} of GA with LOD of 0.03 mg L^{-1}. A sample throughput was 21 injections per hour. For the continuous flow system using FC assay, the optimum condition of a water of carrier solution, a 1:15 v/v of FC in water, a 0.25 mol L^{-1} of NaOH, a 1.6 mL min^{-1} of flow rate, a 20 cm of RL length, a 2:1:1 (s:s:s) of aspiration times of S:FC:NaOH and a 728 nm of detection wavelength, were chosen. A linear calibration graph was 2.5 – 15 mg L^{-1} of GA with LOD of 0.04 mg L^{-1}. A sample throughput was 17 injections per hour.

Both proposed continuous flow systems using ABTS and FC assays were successfully applied for the determination of antioxidant capacity and total phenolic compounds in twenty samples of tea and herbal teas. These samples were Cha keaw (green tea), Cha Keqw Yepun (Japanish green tea), Kheelek, Maa Khee Nok, Yaa Puk King, Takhrai, Cha Em Ted, Chaa Phluu, Krachip Daeng, Mon, Kha Chay Dum,

Khamin Khruea, Thaowan Prieng, Bua Bok, Khing, Chumhet Thet, Makhaam Pom, Matuum, Makhaam Khaek and Yaa Nuat Maeo teas. For the system of ABTS assay, it was established that the antioxidant capacity contents in the teas and herbal teas were found in the range of 56 ± 1 – 5952 ± 202 mg kg^{-1} of dry weight. The higher contents of antioxidant capacity were found in Makhaam Pom, Cha keaw and Cha Keqw Yepun teas while the lower contents were found in Yaa Puk King, Khamin Khruea and Mara Khee Nok teas. For the system of FC assay, the contents of total phenolics compounds were found in range of 216 ± 4 – 9035 ± 287 mg kg^{-1} of dry weight. The higher contents of total phenolic compounds were also found in Makhaam Pom, Cha keaw and Cha Keqw Yepun teas while the lower contents were found in Yaa Puk King, Takhrai and Mara Khee Nok teas. Furthermore, the obtained contents from the two proposed continuous flow systems using both assays were in good agreement with those obtained from the microplate method (t-test of $t_{calculation}$ = 0.14 and 0.38 for ABTS and FC assays and $t_{critical}$ = 2.10, 95% confidence level). The proposed systems offer several advantages, including fast analysis, low reagent and sample consumptions, low waste generation, easy operation, semi-automatic system and acceptable accuracy and precision.

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APPENDICES

APPENDIX A THE ABTS$^{\bullet+}$ AND FOLIN CIOCALTEU' S PHENOL REAGENT SOLUTION IN DIFFERENT MEDIUM SOLUTIONS

The ABTS$^{\bullet+}$ and Folin ciocalteu's phenol reagent (FC) in different medium solutions used in this work were shown in Figure 29. These ABTS$^{\bullet+}$ solutions were 1) a ABTS$^{\bullet+}$ stock solution (7 mmol L^{-1} ABTS + 2.45 mmol L^{-1} $K_2S_2O_8$ + water), 2) a ABTS$^{\bullet+}$ working solution (ABTS stock solution was diluted with water as 0.7 absorbance value) and 3) a mixed solution of ABTS$^{\bullet+}$ + 0.1 mmol GA L^{-1}, and 4) a mixed solution of ABTS$^{\bullet+}$ + 1 mmol GA L^{-1} respectively. For FC assay, All FC solutions were 1) a 1:10, v:v of FC reagent: water, 2) a mixed solution of 0.25 mol L^{-1} of NaOH + FC reagent, and 3) a mixed solution of 0.25 mol L^{-1} NaOH + 1:10, v:v FC reagent + 0.1 mmol GA L^{-1}.

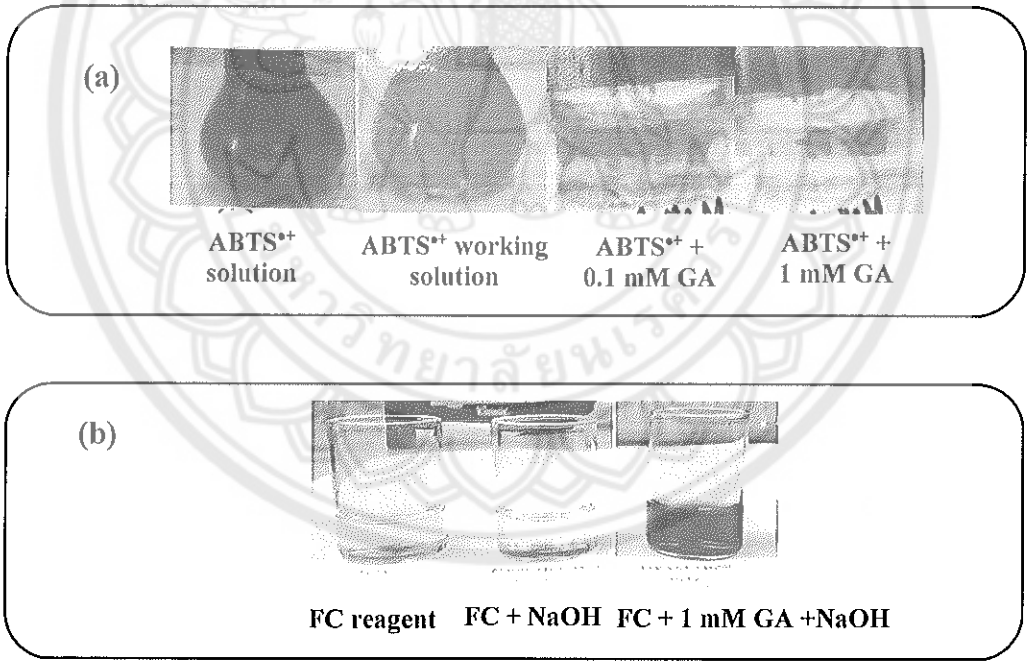


Figure 29 (a) ABTS$^{\bullet+}$ solutions in different medium solutions for the determination of antioxidant capacity and (b) FC solution in different medium solutions for the determination of total phenolic compounds

APPENDIX B THE SAMPLE COLLECTION OF BANANA WASTES AND BANANA EXTRACT NAMES

The schematic diagram of banana (Kluai Nam Wa Mali Ong) waste collection and banana extract names of peel, hand stalk and bunch stalk were described in Figure 30 - 32, respectively. These waste were collected from different provinces, seasons and also its ripeness and then were extracted for the determination of GA, GC, C, EC, and EGCG by HPLC.

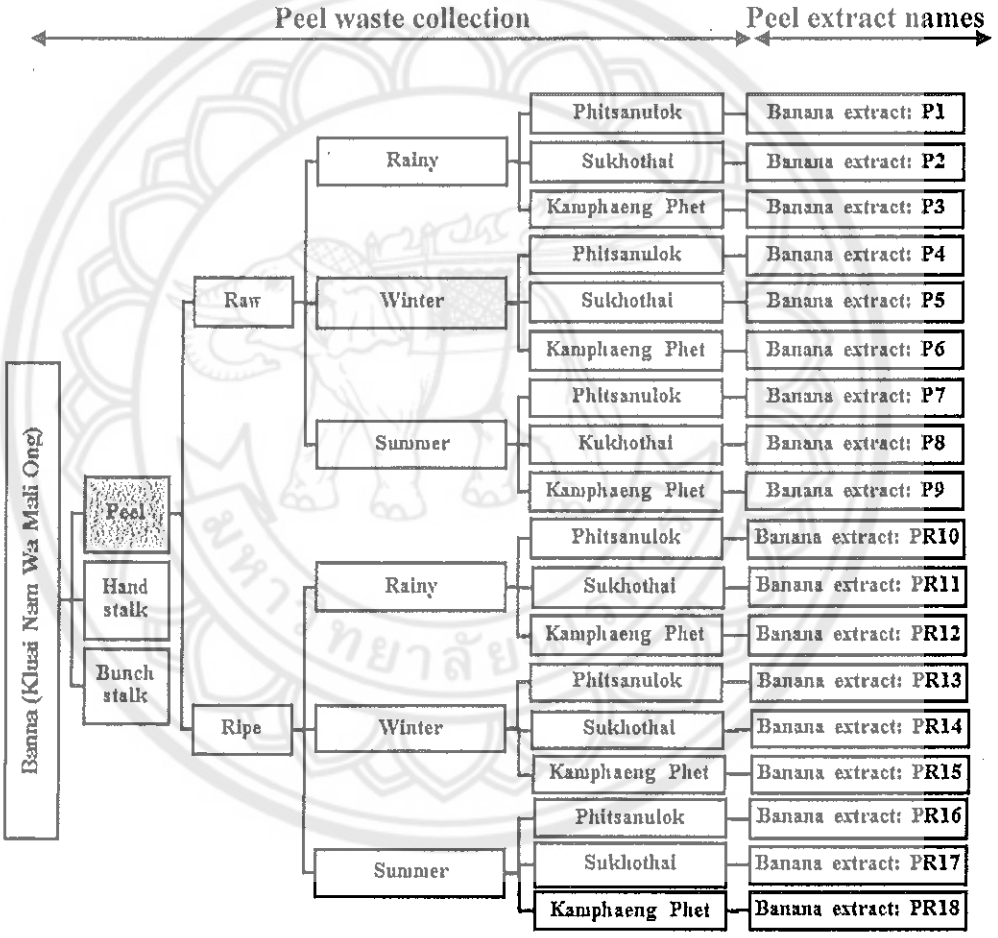


Figure 30 The schematic diagram of banana (Kluai Nam Wa Mali Ong) peel waste collection and names of its extracts from different provinces and seasons

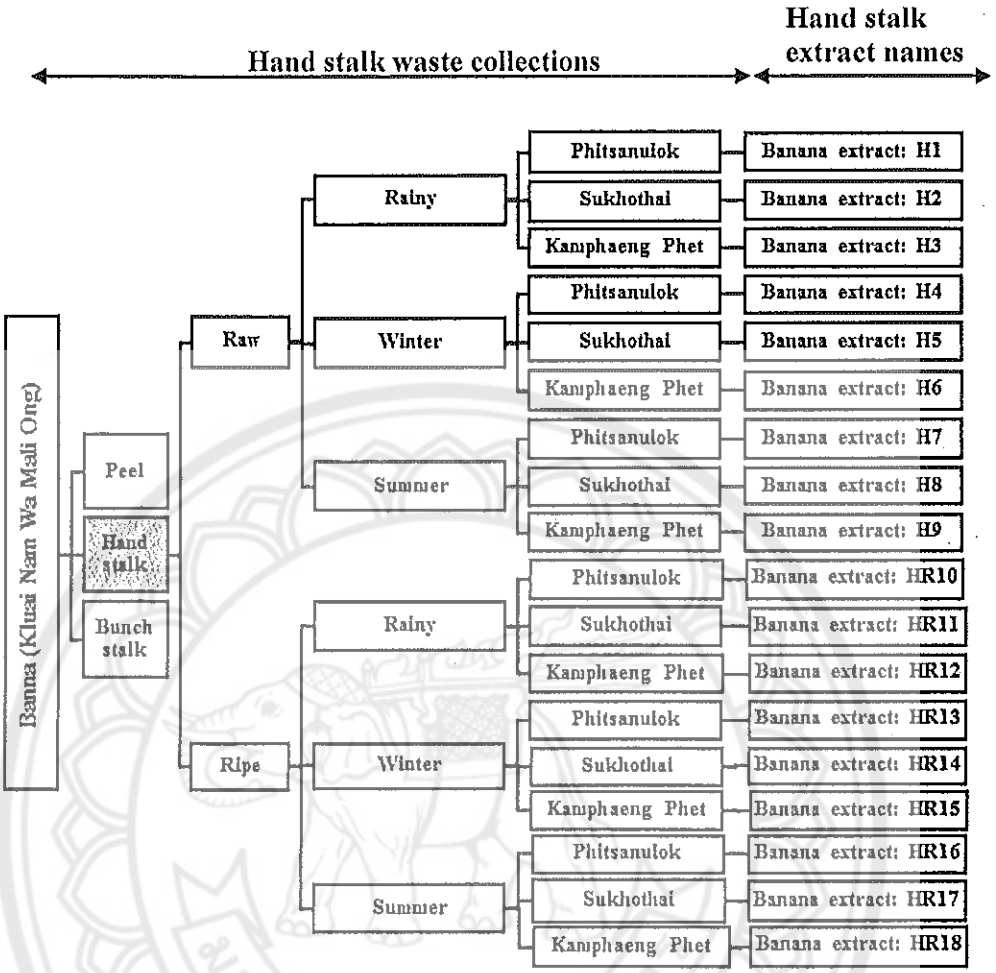


Figure 31 The schematic diagram of banana (Kluai Nam Wa Mali Ong) hand stalk waste collection and name of its extracts from different provinces and seasons

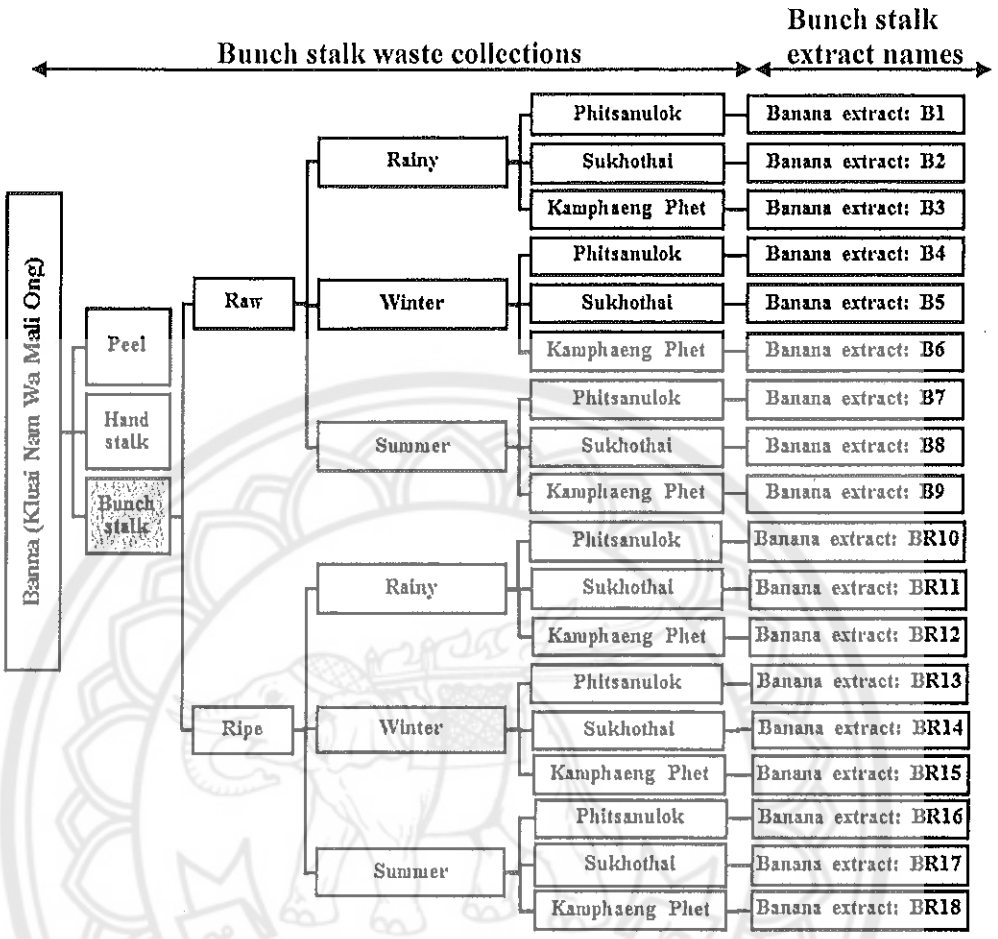


Figure 32 The schematic diagram of banana (Kluai Nam Wa Mali Ong) bunch stalk waste collection and names of its extracts from different provinces and seasons

APPENDIX C THE BOTANICAL CHARACTERISTICS OF SOME THAI HERBS AND TEAS

The botanical characteristics of Thai herbs (produced herbal teas) and teas (Cha-keaw and cha-keaw-yepun) were summarized in Table 34. These Thai herbs were commonly prepared and produced for commercial herbal teas and used in this work.

Table 34 The botanical characteristic of some Thai herbs and teas [98, 99, 100]

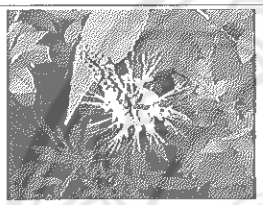
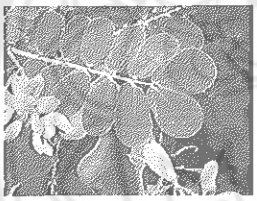
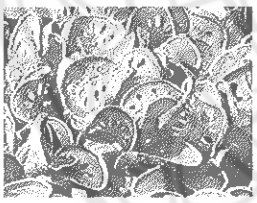
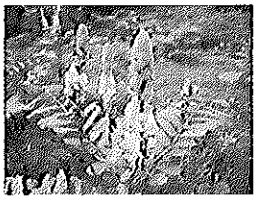
No.	Thai herbs and teas	Botanical characteristics
1		**Thai name:** Yaa Nuat Maeo, **English name:** Cat's whisker **Species:** *Orthosiphon grandiflorus* Boldingh. **Family:** LABIATAE **Part usage:** Leaf **Indication:** Expels urine
2		**Thai name:** Makhaam Khaek, **English name:** Senna **Species:** *Cassia angustifolia* Vahl. **Family:** CAESALPIAIACEAE, FABACEAE **Part usage:** Dried leaf and young pod **Indication:** Used as laxative and stimulate purging
3		**Thai name:** Matuum, **English name:** Bael Fruit **Species:** *Aegle marmelos* (Linn.) Corr. **Family:** RUTACEAE **Part usage:** Ripe fruit **Indication:** Laxative and promotes digestion
4		**Thai name:** Makhaam Pom, **English name:** Indian Gooseberry **Species:** *Phyllanthus emblica* Linn. **Family:** EUPHORBIACEAE **Part usage:** Fruit **Indication:** Relieves of cough and diuretic, expectorant
5		**Thai name:** Chumhet Thet, **English name:** Ringworm Bush **Species:** *Cassia alata* Linn. **Family:** CAESALPINIACEAE, FABACEAE **Part usage:** Dried leaf and fresh flower **Indication:** Used as laxative

Table 34 (cont.)

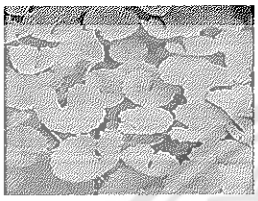
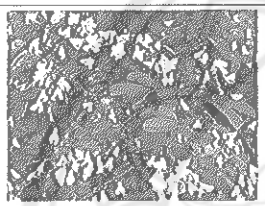
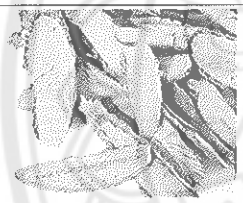
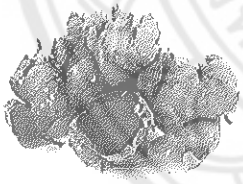
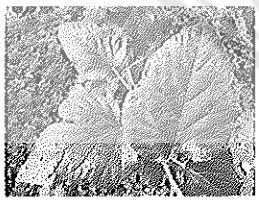
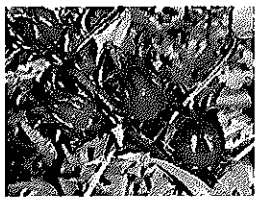
No.	Thai herbs and teas	Botanical characteristics
6		**Thai name:** Khing, **English name:** Ginger **Species:** *Zingiber officinale* Rosc. **Family:** ZINGIBERACEAE **Part usage:** Rhizome **Indication:** Carminative, antiemetic and expectorant
7		**Thai name:** Bua Bok, **English name:** Asiatic Pennywort **Species:** *Centella asiatica* (Linn.) Urban **Family:** UMBELLIFERAE **Part usage:** Leaf **Indication:** Antipyretic, diuretic and antidiarrheal
8		**Thai name:** Thaowan Prieng, **English name:** Jewel Vine **Species:** *Derris scandens* (Roxb.) Benth. **Family:** LEGUMINOSAE **Part usage:** Stem **Indication:** Diuretic and antidysenteric
9		**Thai name:** Khamin Khruea, **English name:** - **Species:** *Arcangelisia flava* (Linn.) Merr. **Family:** MENISPERMACEAE **Part usage:** Wood and root **Indication:** Blood tonic and antimalarial
10		**Thai name:** Kha Chay Dum, **English name:** Kaempfer **Species:** *Kaempferia parviflora* Wallich. ex Baker. **Family:** ZINGIBERACEAE **Part usage:** Rhizome **Indication:** Tonic and carminative
11		**Thai name:** Mon, **English name:** White Mulberry **Species:** *Morus alba* Linn. **Family:** MORACEAE **Part usage:** Leaf **Indication:** Relief of cough, sedative and decoction
12		**Thai name:** Krachiap Daeng, **English name:** Roselle **Species:** *Hibiscus sabdariffa* Linn. **Family:** MALVACEAE **Part usage:** Sepal **Indication:** Diuretic

Table 34 (cont.)

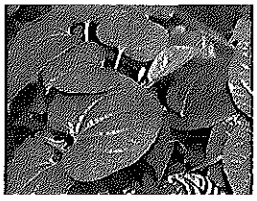
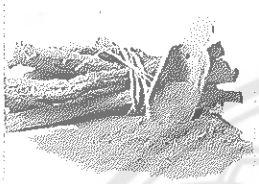
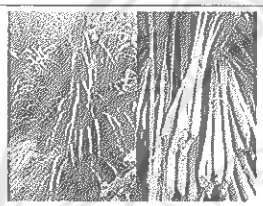
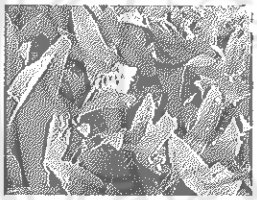
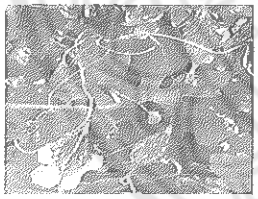
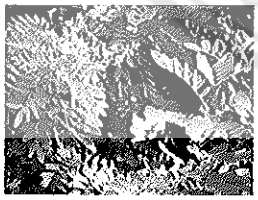
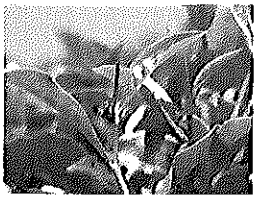
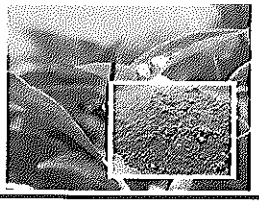
No.	Thai herbs and teas	Botanical characteristics
13		**Thai name:** Chaa Phluu, **English name:** - **Species:** *Piper sarmentosum* Roxb. ex Hunter **Family:** PIPERACEAE **Part usage:** Leaf **Indication:** Blood glucose lowering
14		**Thai name:** Cha Em Ted, **English name:** Spanish Licorice **Species:** *Glycyrrhiza glabra* Linn. Var. *typical* Regel. **Family:** LEGUMINOSAE **Part usage:** Root **Indication:** Cures coughing and expels phlegm
15		**Thai name:** Takhrai, **English name:** Lemon Grass **Species:** *Cymbopogon citratus* (DC.) Stapf. **Family:** GRAMINAE **Part usage:** Leaf sheath and rhizome **Indication:** Carminative
16		**Thai name:** Yaa Puk King, **English name:** - **Species:** *Murdannia loriformis* (Hassk.) Rolla Rao et Kammathy **Family:** COMMELINACEAE **Part usage:** Whole plant **Indication:** Cures lymphatic disorders
17		**Thai name:** Mara Khee Nok, **English name:** Bitter cucumber **Species:** *Momordica charantia* Linn. **Family:** CUCURBITACEAE **Part usage:** Fruit and leaf **Indication:** Anthelmintic and antipyretic
18		**Thai name:** Kheelek, **English name:** Cassod Tree **Species:** *Cassia siamea* Britt. **Family:** LEGUMINOSAE, FABACEAE **Part usage:** Leaf **Indication:** Used as laxative and diuretic
19		**Thai name:** Cha-Keaw, **English name:** Chinese green tea **Species:** *Camellia sinensis* **Family:** THEACEAE **Part usage:** Leaf and leaflet **Indication:** Used as antioxidant

Table 34 (cont.)

No.	Thai herbs and teas	Botanical characteristics
20		**Thai name:** Cha-Keaw-Yepun, **English name:** Japanese green tea **Species:** *Camellia sinensis* **Family:** THEACEAE **Part usage:** Leaft and leaflet **Indication:** Used as antioxidant