# CHAPTER 1

# Introduction

## 1.1 Tea

Tea, *Camellia sinensis* is a species of flowering plants in the genus *Camellia* and the family Theaceae. It is the most widely consumed beverage in the world and has been an important agricultural product.[1] Recent studies have shown tea confers great beneficial effects to the health of consumers, including the effects of reduction cholesterol, enhancing insulin activity, imunostimulatory, antimicrobial, antioxidation activities, protection against cardiovascular disease and cancer, postulating to have antimutagenic and anticarcinogenic properties and used as topical applications for woud-healing, anti-aging and disease treatments.[2-3] There are two varieties of *C. sinensis* as follows:

*Camellia sinensis* var. *sinensis* (China tea or green tea) is grown extensively in China, Japan and Taiwan. It is an evergreen shrub or tree and can grow to heights of 30 feet, but is usually pruned to 2-5 feet for cultivation. The leaves are dark green, alternate and oval, with serrated edges, and the blossom are white, fragrant, and appear in clusters or singly.

*Camellia sinensis* var. *assamica* (Assam tea) is distributed in the South and Southeast Asia, including the northern part of Thailand.[4] It is characterized by tea leaves larger than China tea. It has a single stem, which grows to be about 6-18 meters in height, grows fast, flowers in a bouquet of 2-4 Assam tea. Figure 1.1 showed the Assam tea plant

A B

**Figure 1.1** *C. sinensis* plant (A) China tea and (B) Assam tea (Sourec; http://www.mfu.ac.th/school/agro2012/node/284)

Processed tea leaves were classified into three types, in respect of different fermentation degree; non-fermented (green), semi-fermented (oolong) as well as entirely fermented (black).[5] The manufacturing process of each type is shown in Figure 1.2.

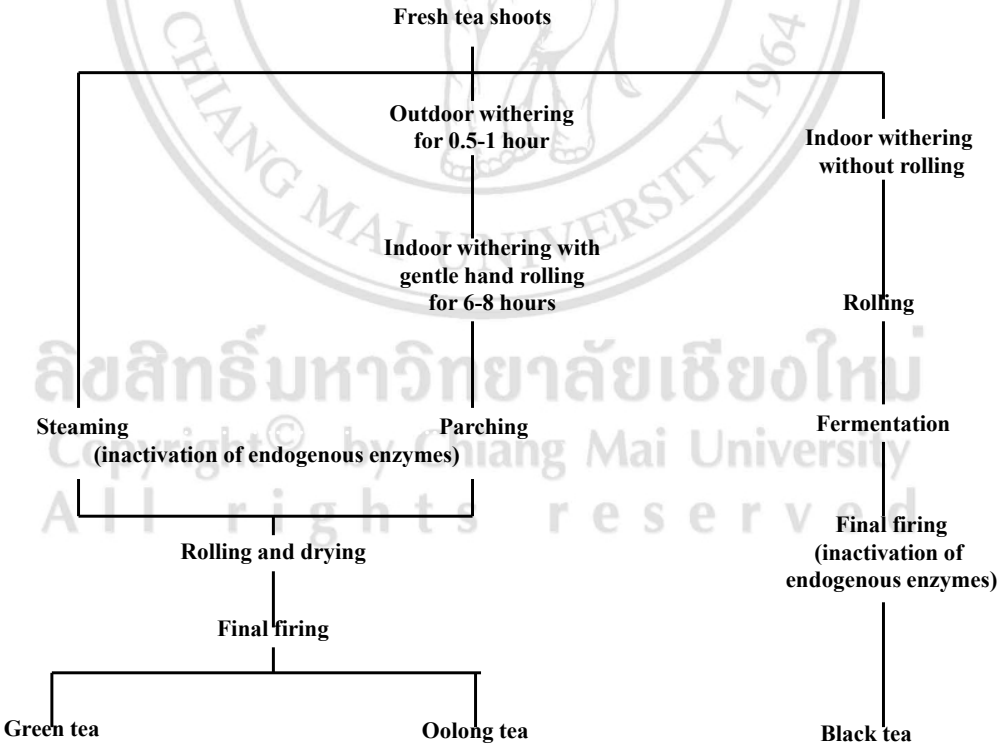




**Figure 1.2** An outline of the manufacturing process for green tea, oolong tea and black tea

Non-fermented teas are known as green tea, white tea and yellow tea which is very rare. The leaves are simply picked, dried and then packaged. The leaves may be steamed, flattened, twisted or rolled into little balls to accentuate the tea's fragrance or taste. This process permits little oxidization of the leaves and retains the fresh taste of the leaves. Contrary to popular belief, Chinese green teas may contain more caffeine than Chinese black teas but both are less than the black teas consumed in the West that originate from India. Howevere, all teas contain significantly less caffeine.[6]

Semi-fermented teas are known as oolong teas that are sometimes called semi-green or blue-green tea. The process is the same as non-fermented teas with the time of oxidization adjusted to make the tea taste more green or black and bring out certain taste characteristics.[7]

Fermented teas are known as black teas (known as red teas in China). The flavour is developed by keeping the leaves warm and moist for a few hours which turns them black as the chemicals in the leaves break them down by reacting with the air. The leaves are then dried by a variety of methods which affects the taste and then graded and pacakaged.[8]

### 1.2 Chemical composition in tea leaves

The chemical components of tea leaves include polyphenols, alkaloids, volatile oils, polysaccharides, amino acids, lipids, vitamins, and inorganic elements. The fresh tea leaves contain caffeine (approximately 3.5% of the total dry weight), theobromine (0.15-0.2%), theophylline (0.02-0.04%) and other methylxanthines, lignin (6.5%), organic acids (1.5%), chlorophyll (0.5%) and other pigments, theanine (4%) and free amino acids (1.0-5.5%), and numerous flavour compounds.[9] Several researchers reported that the chemical constituents of various kinds of tea leaves (green tea, black tea and oolong) are as follows.

The chemical composition of green tea consists phenolic compounds and triterpene saponins, and foliatheasaponin. The major component include (+)-catechin (C, **1**), (−)-epicatechin (EC, **2**), (−)-epigallocatechin (EGC, **3**), (−)-epigallocatechin 3-*O*-gallate (EGCG, **4**), (−) epicatechin 3-*O*-gallate (ECG, **5**), (−)-epigallocatechin 3-*O*-*p*-coumaroate (**6**), (−)-epigallocatechin 3,3'-di-*O*-gallate (**7**), (−)-epigallocatechin 3,4'-di-*O*-gallate (**8**), prodelphinidin B-2 3'-*O*-gallate (**10**), procyanidins B-2 3,3'-di-*O*-gallate (**9**), procyanidins

B-2 3'-*O*-gallate (**11**), procyanidins B-4 3'-*O*-gallate (**12**), (–)- epicatechin 3-*O*-(3-*O*-methyl)-gallate (**13**), theasinensins A (**14**), theasinensins B (**15**), (–)-catechin gallate (CG, **16**), (–)-gallocatechin (GC, **17**), (–)-epigallocatechin 3-*O*- (3′′-*O* methyl) gallate (**18**), (–)-epigallocatechin 3,5-di-*O*-gallate (**19**), 1,4,6-tri-*O*-Galloyl-*β*-D-glucose (**20**) , foliatheasaponin I (**21**), foliatheasaponin II (**22**), foliatheasaponin III (**23**), foliatheasaponin IV (**24**), foliatheasaponin V (**25**), (–)-gallocatechin gallate; GCG (**26**), caffeine (**27**), theobromine (**28**) and theophylling (**29**).[10-12] The major volatile components identified from green tea were *(E)*-2-hexanal (**30**), hexanal (**31**), heptanal (**32**), pentanal (**33**), hexanol (**34**), *(E)*-2-hexanol (**35**), *(Z)*-3-hexanol (**36**), *(E)*-geraniol (**37**), nerolidiol (**38**), linalool (**39**) and linalool oxides (**40**).[13-15]

The chemical composition of oolong tea has proanthocyanidins, hydrolysable tannin, red pigments, flavan-3-ol, and dimeric flavan-3-ols, including theasinensins A , theasinensins B, theasinensin C (**41**), theasinensin D (**42**), theasinensin E (**43**), theasinensin F (**44**), theasinensin G (**45**), oolongtheanin (**46**), EGC, ECG, EGCGoolonghomobisflavans A (**47**), oolonghomobisflavans B (**48**), 8-*C*-ascorbyl EGCG (**49**), EC-(4*β*→8)-EGCG (**50**), ECG-(4*β*→8) EGCG (**51**), C-(4*α*→8)-EGCG (**52**), ECG-(4*β*→6)-EGCG (**53**), EGCG-(4*β*→6)-ECG (**54**), epiafzelechin 3-*O*-gallate-(4*β*→6)-EGCG (**55**), EGC-(4*β*→8)-ECG (**56**), EGCG-(4*β*→8)-ECG (**57**), EGC-(4*β*→8) ECG [prodelphinidin B-2 3'-*O*-gallate] (**58**), EGCG-(4*β*→8)-EGCG-[prodelphinidin B-2 3,3'-di-*O*-gallate] (**59**), C-(4*β*→8)-EGC (**60**), GC-(4*β*→8)-EC (**61**), prodelphinidin B-4 (**62**), EGCG-(4*β*→6)-EGCG[prodelphinidin B-5 3,3'-di-*O*-gallate] (**63**), procyanidin B-2 (**64**), procyanidin B-3 (**65**), procyanidin B-4 (**66**), procyanidin B-5 3,3'-di-*O*-gallate (**67**), theaflavin (**68**), theaflavin 3-*O*-gallate (**69**), theaflavin 3'-*O*-gallate (**70**), theaflavin 3,3'-di-*O*-gallate (**71**), theogallin (**72**), *β*-glucogallin (**73**) and strictinin (**74**).[16-17]The main volatile constituent of oolong tea were nerolidol (**75**), indole (**76**), benzeneacetaldehyde (**77**), linalool, linalool oxides, hexanal, *(E)* geraniol (**78**), 1-penten-3-ol (**79**), methyl salicylate (**80**), methyl jasmonate (**81**), phenylethyl alcohol (**82**), benzyl alcohol (**83**), *(Z)*-jasmone , *β*-ionone (**84**), 3,7-dimethyl-1,5,7 octatrien-3-ol, 2,5-dimethyl pyrazine (**85**), 5-methyl-2-furancarboxaldehyde (**86**) and isoeugenol (**87**).[18-21]

The chemical composition of black tea consists novel chalcan-flavan dimers, flavan-3-ol, proantrocyanindins, theasinensins and hydrolzable tannins. The main

compounds include EGCG, EC, C, GC, EC, EGC, (–)-epiafzelechin (**88**), (–)-epiafzelechin 3-*O*-gallate (**89)**, (–)-epicatechin 3,5'-di-*O*-gallate (**90**), (–)-epigallocatechin-*O*-*p*-coumaroate, (–)-epigallocatechin 3,5'-di-*O*-gallate, procyanidin B-3, procyanidin B2 3,3'-di-*O*-gallate EGCG-(4*β*-8)-ECG, prodelphinidin B-2 3,3'-di-*O*-gallate, theaflavin, prodelphinidin B-4, prodelphinidin B-4 3'-*O*-gallate, procyanidin C-1 (**91**), theogallin, 1,4,6-Tri-*O*-galloyl-*β*-*O*-glucose, strictinin, theasinensin A, theasinensin B, gallic acid (GA, **92**), theaflavin 3-*O*-gallate, theaflavin 3'-*O*-gallate, theaflavin 3,3'-di-*O*-gallate, caffeine, assamicain A (**93**), assamicain B (**94**), assamicain C (**95**), (–)-epigallocatechin-3-*O*-caffeoate (**96**), prodelphindin B-4, desgalloyl theasinensin F (**97**), 1-*O*-galloy-4,6-(*S*)-hexahydroxydiphenoyl-*β*-D-glucopyranose (**98**), Epitheaflagallin 3-*O*-gallate (**99**), epitheaflagallin (**100**) and theaflagallin (**101**).[22-23] The volatile compounds were major compound including (*E*)-2-hexenal, benzaldehyde (**102**), benzeneacetaldehyde, (*E*)-geraniol, nerolidiol, linalool, linalool oxides, methyl salicylate, (*Z)*-3-hexen-1-ol (**103**), *β*-ionone, furfural, (**104**), *(E,E)*-2,4-decadienal (**105**), *(E,E)*-2,4-hexadienal (**106**), *(E)*-2-hexen-1-ol (**107**) and *(E)*-*β*-damascenone (**108**).[24-29] The major compounds of Green tea, oolong, and black tea were catechins compounds (compound 1, 2, 3, 4, 5, 16, 17 and 34).

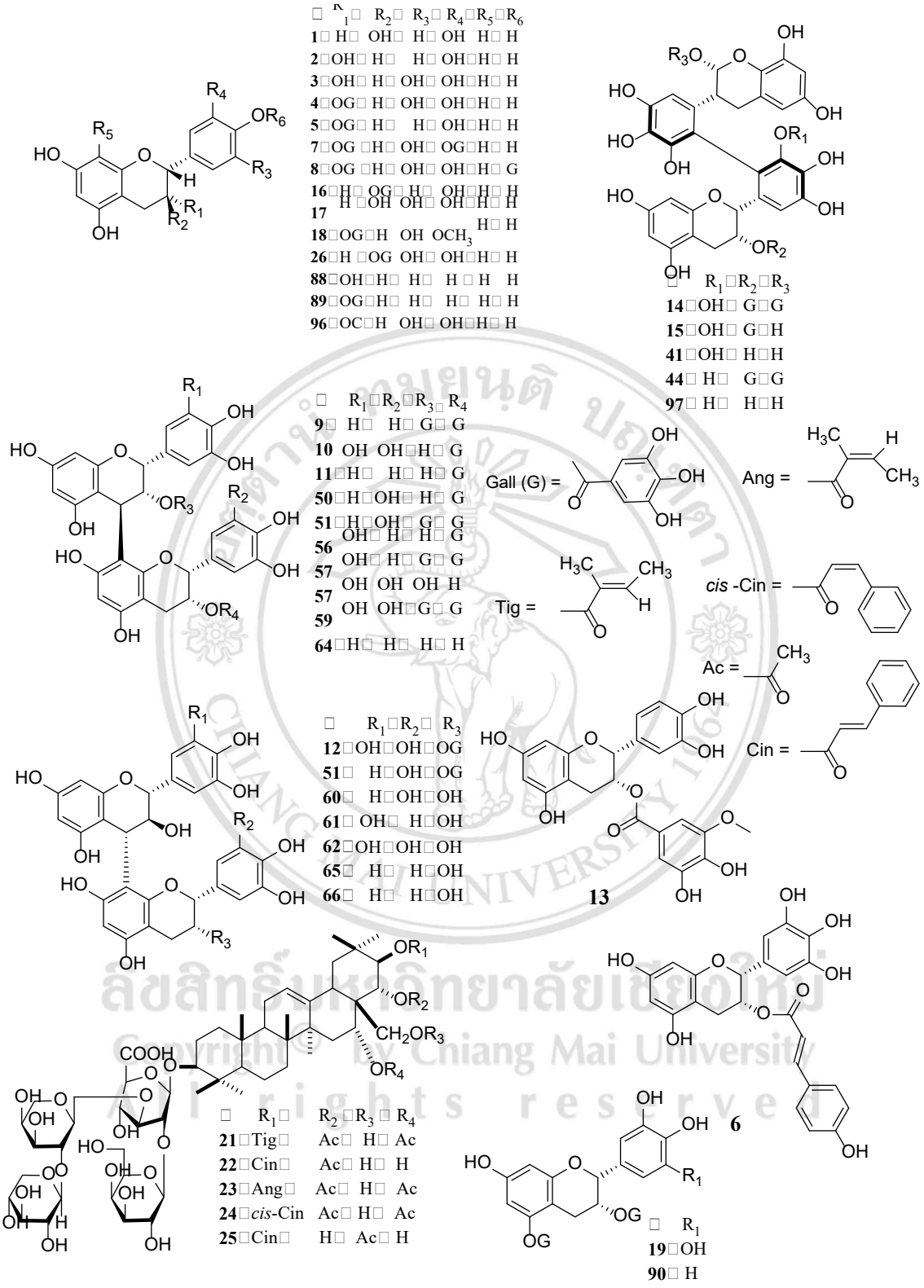


**Figure 1.3** Structure of chemical compound from Assam tea

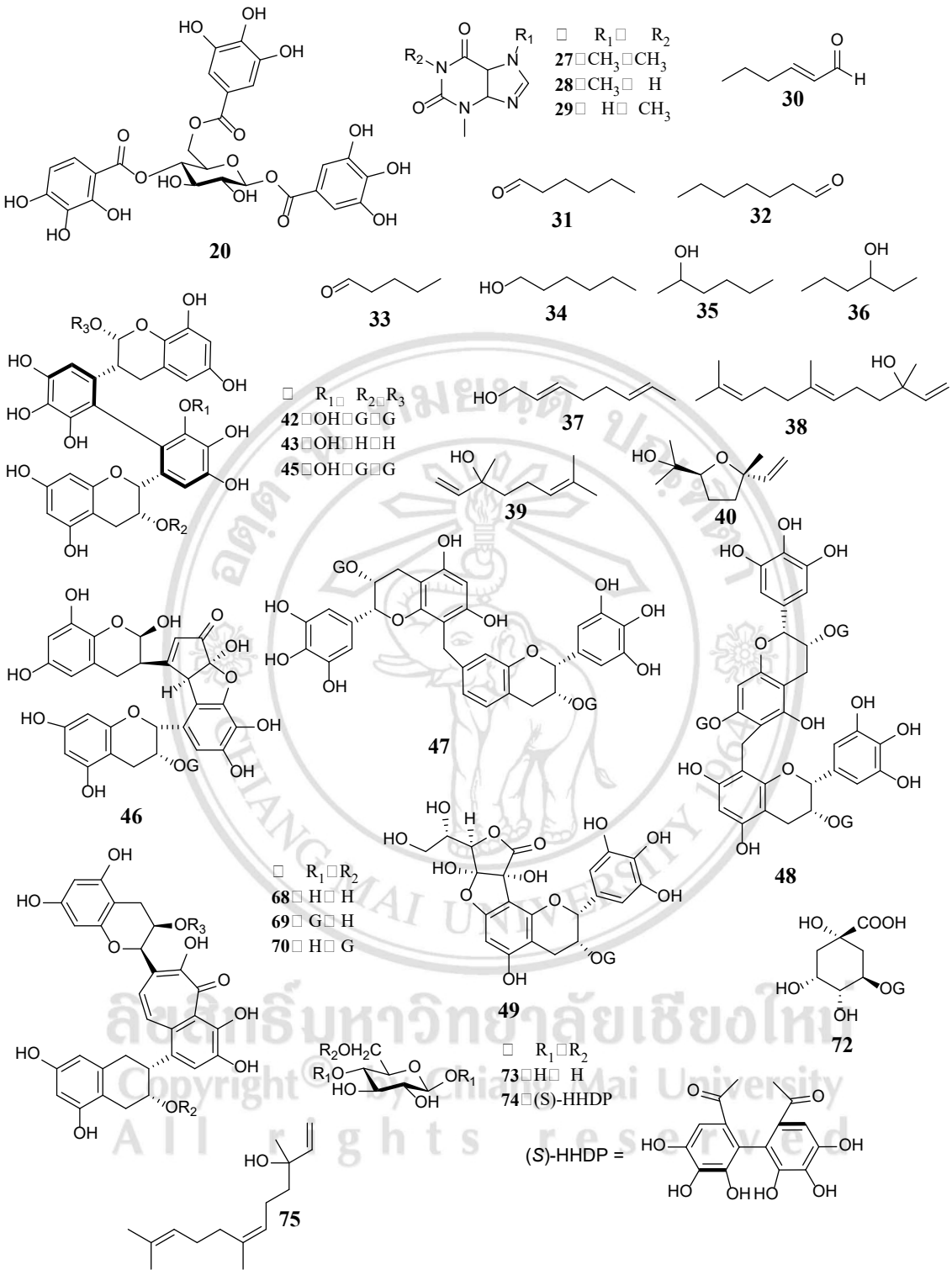


**Figure 1.3** Structure of chemical compound from Assam tea (continuous)

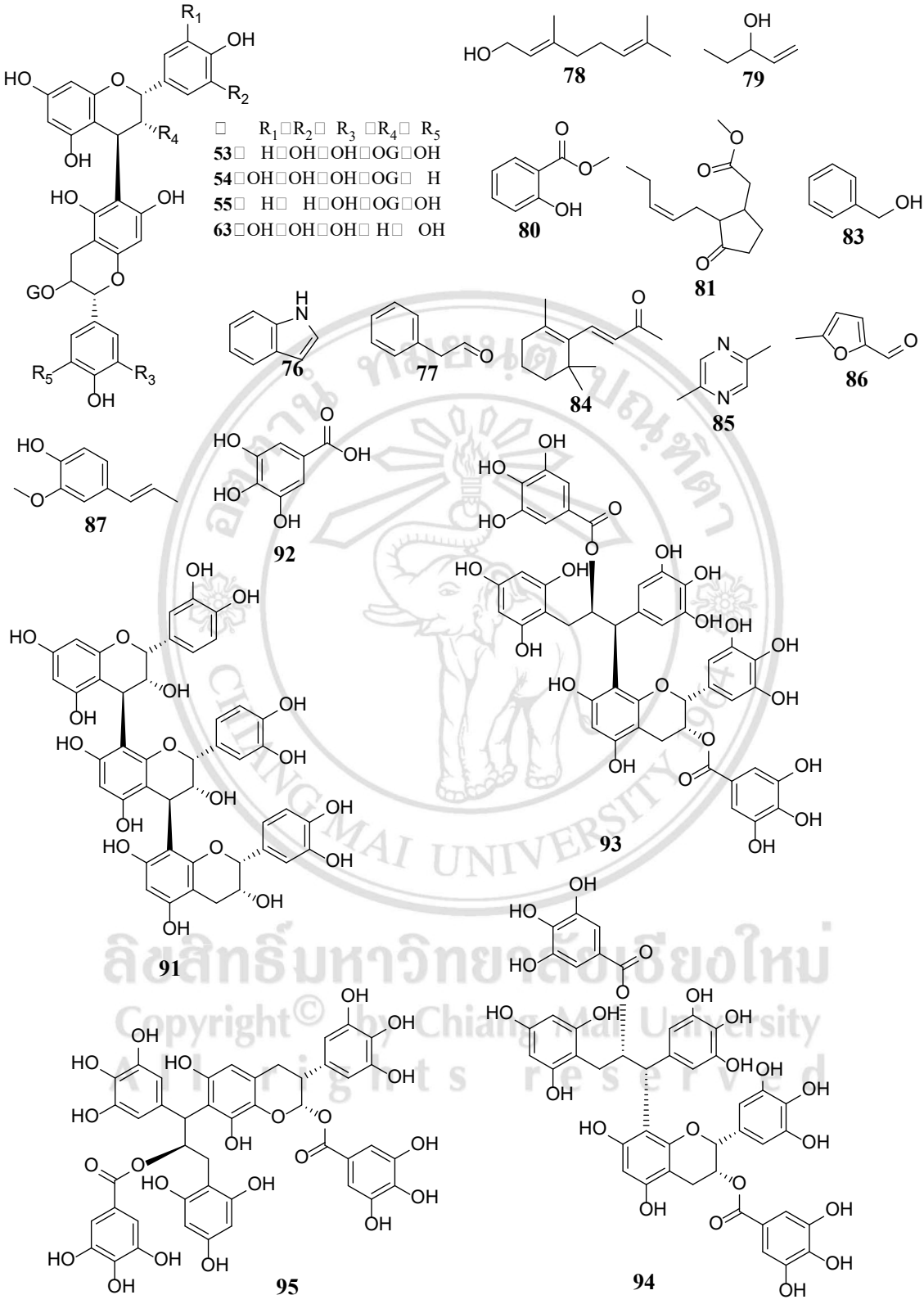


**Figure 1.3** Structure of chemical compound from Assam tea (continuous)

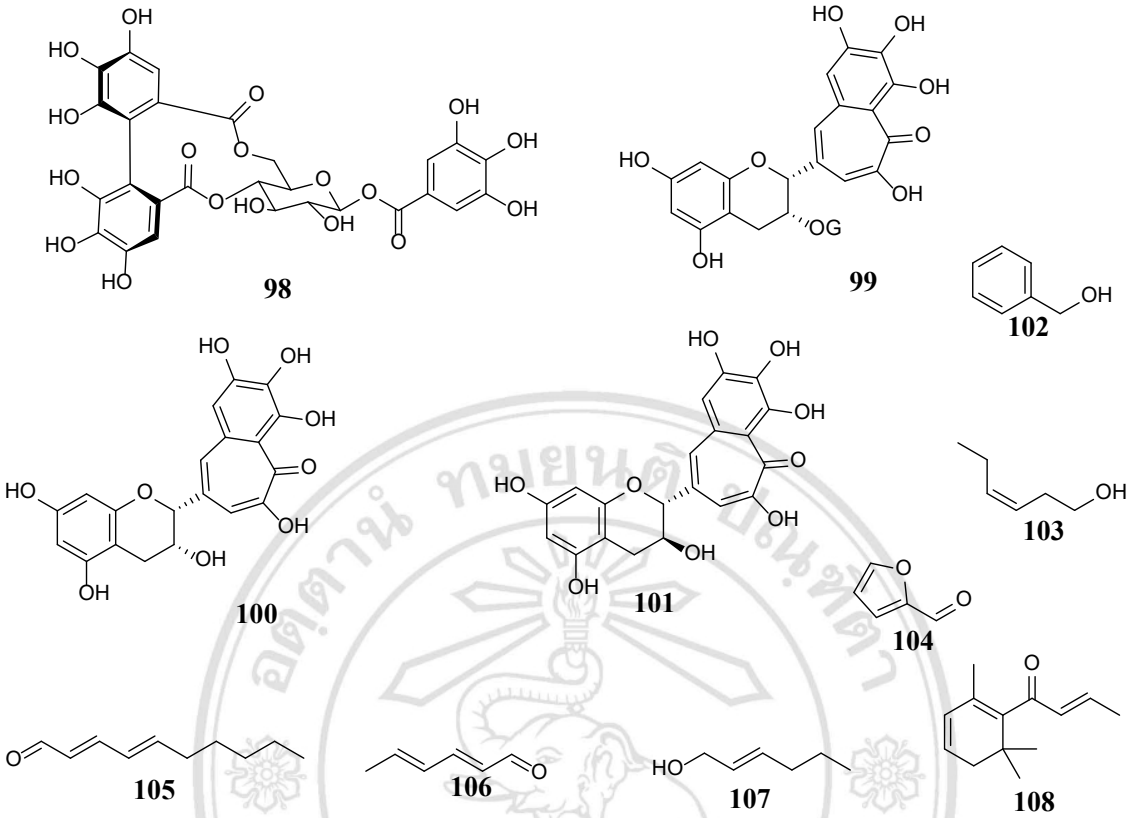


**Figure 1.3** Structure of chemical compound from Assam tea (continuous)



## 1.3 Miang (Fermented tea)

Miang is comprised of non-salted, fermented or pickled tea leaves that are traditionally produced in the mountainous areas of the northern Thailand.[30] It can be consumed in form of snack, guest welcoming snack, or used in various northern ceremonies. Fresh tea leaves of *Camellia sinensis* var. *assamica* are conventionally used as the raw material for Miang fermentation. This tea plant is commonly called "Miang tree" by northern Thai people and it can be found throughout Chiang Mai Province as well as in other upper northern provinces of Thailand, including Lampang, Chiang Rai, Phayao, Nan and Phrae with elevations as high as 450-1500 m above sea level. However, the tea plant is most commonly found at heights of 600-1000 m above sea level.[31] Miang cultivation is achieved by seed culture and seedlings are usually planted in the intervening space between two large trees. Miang culture thus has a relatively benign effect on the environment. There is no need to apply fertilizer and maintenance involves occasional trimming and weed removal.[32] Miang can be classified by local people of northern Thailand, including astringency Miang, Miang produced from young tea leaf. It is tied and streamed then taking or fermented immediately after streamed in order to be kept longer without become sour, and sour Miang (Miang Prew), which Miang made by steamed tea leaf, overnight left, and then fermented until it become sour taste which is ready for consumption. Phenolic compounds, catechin particularly, are a group of main astringent and bitter substance in tea and contain obvious antioxidant property.[33]

The manufacturing process of Miang includes many steps as shown in Figure 1.4. Young tea leaves are collected, then tied into bundles with bamboo strip, and steamed in big wooden bucket for 2 hours or more to inactivate the polyphenol oxidases, and then spread out to cooling down, after that steamed leaf is re-tied into new bundles which are carefully packed in a bamboo basket lined with banana leaves, and covered with banana leaves or plastic sheet. The steamed leaves are pressed tightly and weighted down to exclude oxygen, and thus to discourage the growth of yeasts and aerobic spoilage bacteria for fermentation process. Fermentation takes about 3 months or longer depending on the desired attributes and Miang is re-packed before sale to customers. The product has a pickled flavor, sour and flowery.[30, 34]

Collected tea leaves

Steamed tea leaves for 2 hr

Spread out to cooling down

Fermented

Packing

(Source; http://nattpong1234.blogspot.com.)

**Figure 1.4** The manufacturing process of Miang

### 1.4 Antioxidant activity

Antioxidants are specific organic compounds that can inhibit the single oxygen molecules or free radical in the body by prevent the oxidation, which is a redox chemical reaction that transfers unpaired electrons in substance to an oxidizing agent. The oxidation involves chain reaction of the production dangerous free radicals and this reaction can stabilize themselves by oxidizing other molecules, including protein, carbohydrates, lipids and DNA.[35] Examples of reactive oxygen species (ROS) or free radicals are singlet oxygen, superoxide anion, hydroxyl radicals, hydrogen peroxide, hypochlorite ion and peroxynitrite, which are major cause of various chronic and degenerative diseases, including ageing, coronay heart disease, stroke, inflammation, diabetes mellitus, dementia and cancer. The oxidative reaction can be finished by antioxidant, which can remove radical intermediates and inhibit the oxidation reaction by being oxidized themselves.[36-37]

Antioxidants are obtained from natural sources, especially from food of plant origin. Fruits, vegetable, herbs, grain cereals and medicinal plants are good natural sources of antioxidants that prevent free radical in the human body. These antioxidants are classified into two broad division, depending on whether they are soluble in water (hydrophilic) or lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytoplasm and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation.[38] Some vitamin and phenolic compounds are known as hydrophilic antioxidant, however carotenoid are known as lipophilic antioxidant as shown in Table 1.1. In recent years, phenolic compounds have attracted the interest of researchers because they showed promise of being powerful antioxidants that can protect the human body from free radicals.



**Table 1.1** Antioxidant compounds found in various foods.[39]

| Antioxidant compounds | Food containing high levels of antioxidants |
|---|---|
| Vitamin C (ascorbic acid) | Fruits and vegetables |
| Vitamin E (tocopherols, tocotrienols) | Vegetable oils |
| Polyphenolic antioxidant (flavonoids) | Tea, coffee, soy, fruits, chocolate and red wine |
| Carotenoids (lycopene, carotenes) | Fruits and vegetables |

Phenolic compounds are important components of many fruits, vegetables and beverages to which they contribute to flavor, color and sensory properties such as astringency. There are a wide range of compounds that possess an aromatic ring bearing a hydroxyl substituent, including their functional derivatives such as esters, methyl esters and glycosides.[40] The major classes of plant polyphenols are listed in Table 1.2 which phenol is the simple structure of the class.

The antioxidant activity of phenolic compounds is mainly due to their ability to scavenge free radicals, donate hydrogen atoms or chelate metal cations, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.[41] Health-related effects of phenolic compounds such as antibacterial, antimutagenic, anticarcinogenic and vasodilatory activities have been reported.[42-45]

Several methods are used to measure the antioxidant activity of a biological material. The most commonly used ones are those involving chromogen compounds of radical nature that stimulate the reductive oxygen species. The method of antioxidant activities measurement of natural products are evaluated including the 2,2'-azinobis (3-ethyl-bezothiazoline-6-sulfonic acid) (ABTS) assay, 2,2-diphenyl-1-picryhydrazyl (DPPH) assay, oxygen radical absorption capacity (ORAC) assay, ferric reducing antioxidant power (FRAP) assay, hydrogen peroxide assay and lipid peroxidation assay.

**Table 1.2** The major classes of phenolic compounds in plants[46]

| Number of carbon atoms | Basic skeleton | Class | Examples |
|---|---|---|---|
| **6** | $C_6$ | Simple phenols<br>Benzoquinones | Catechol, hydroquinone<br>2,6-Dimethoxybenzoquinone |
| **7** | $C_6$-$C_1$ | Phenolic acids | Gallic acid, salicylic acid |
| **8** | $C_6$-$C_2$ | Acetophenones<br>Tyrosine derivatives<br>Phenylacetic acids | 3-Acetyl-6-methoxybenzaldehyde<br>Tyrosol<br>p-Hydroxyphenylacetic acid |
| **9** | $C_6$-$C_3$ | Hydroxycinnamic acids<br>Phenylpropenes<br>Coumarins<br>Isocoumarins<br>Chromones | Caffeic acid, ferulic acid<br>Myristicin, eugenol<br>Umbelliferone, aesculitin<br>Bergenon<br>Eugenin |
| **10** | $C_6$-$C_4$ | Naphthoquinones | Juglone, plumbagin |
| **13** | $C_6$-$C_1$-$C_6$ | Xanthones | Mangiferin |
| **14** | $C_6$-$C_2$-$C_6$ | Stilbenes<br>Anthraquinones | Resveratrol<br>Emodin |
| **15** | $C_6$-$C_3$-$C_6$ | Flavonoids<br>Isoflavonoids | Quercetin, cyanidin<br>Genistein |
| **18** | $(C_6$-$C_3)_2$ | Lignans<br>Neolignans | Pinoresinol<br>Eusiderin |
| **30** | $(C_6$-$C_3$-$C_6)_2$ | Biflavonoids | Amentoflavone |
| **n** | $(C_6$-$C_3)_n$<br>$(C_6)_n$<br>$(C_6$-$C_3$-$C_6)_n$ | Lignins<br>Catechol melanins<br>Flavolans<br>(Condensed Tannins) | Lignins<br>Catechol melanins<br>Flavolans<br>(Condensed Tannins) |

### 1.4.1 Total phenolic contents assay

The total phenolic contents are measured using the Folin-Ciocalteu method.[47] This method is based on the oxidation of phenolic groups with Folin-Ciocalteu reagent (phosphomolybdic and phosphotungstic acids). The chemistry behind the Folin-Ciocalteu reagent assay counts on the transfer of electrons in alkaline medium from phenolic compounds and other reducing species to molybdenum, forming blue complexes that can be monitored spectrophotometrically at 750-765 nm by spectrophotometer as shown in equation 1.[48] The phenolic compounds react with Folin-Ciocalteu reagent only under basic conditions (adjusted by a sodium carbonate solution to pH~10).[49]

$$\text{Phenolic compound} + \text{Folin reagent} + Na_2CO_3 \longrightarrow \text{Blue-green complex} \quad (1)$$

The method is simple, sensitive and precise. The total phenolic contents are determined by comparison with standard calibration curve of gallic acid and expressed as milligrams (mg) of gallic acid equivalents (GAE) per gram dried weight.

### 1.4.2 DPPH radical scavenging activity assay

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts.[50] 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep purple color, characterized by an absorption band in ethanol solution centered at about 517 nm.[51] The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant as shown in Figure 1.5.

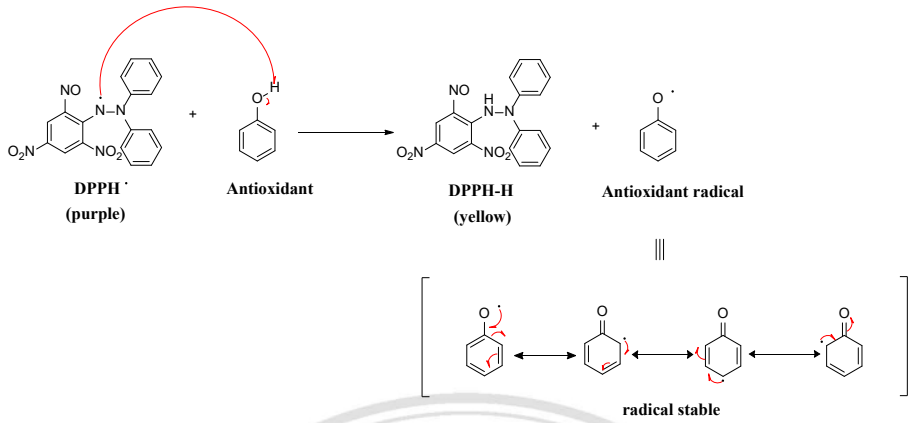


**Figure 1.5** Reaction of DPPH· with antioxidant

The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl group and positive correlated with total phenolic content. The results have also been reported as $IC_{50}$ value, which is the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%.

### 1.4.3 Ferric ion reducing antioxidant power assay (FRAP)

The reducing power of bioactive compounds is associated with their electron donating capacity and this is reflected with their antioxidant activity.[52] Inactivation of oxidants by reductants (antioxidants) can be described as redox reactions in which one reaction species is reduced at the expense of the oxidation of the other. The presence of reductants such as antioxidant substances in the samples causes the reduction of the potassium ferricyanide ($Fe^{3+}$) complex to the ferrous form as shown in Figure 1.6. Therefore, $Fe^{2+}$ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.[53] In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[54] The higher absorbance value indicates a stronger reducing power of sample.

$$Fe(CN)_6^{3-} + \text{antioxidant} \rightleftharpoons Fe(CN_6)^{4-} + \text{oxidized antioxidant}$$

$$Fe(CN)_6^{4-} + Fe^{3+} \rightleftharpoons Fe[Fe(CN)_6]^{-}$$

**Figure 1.6** Reaction of FRAP assay

### 1.4.4 Antioxidant activity of tea leaves

Tea polyphenols, mainly flavonoids, are well-known for their antioxidant properties. The antioxidant activity of tea polyphenols is primarily attributed to the combination of aromatic rings and hydroxyl groups that assemble their chemical structure and consequently binding and neutralization of lipid free radicals by these hydroxyl groups. Numerous studies have demonstrated that polyphenols and tea catechins are exceptional electron donors and effective scavengers of physiologically relevant reactive oxygen species *in vitro*, including superoxide anions, peroxyl radicals, and singlet oxygen.[55-59]

The antioxidant activity of ethanol and water extracts of green and black tea, was measured by two different methods of scavenging; the stable free radicals $ABTS^{+\bullet}$ and $DPPH^{\bullet}$.[60] The examination of extracts showed different antiradical activities. The best activity in scavenging $ABTS^{+\bullet}$ expressed as TAA (total antioxidant activity) was reported for black tea aqueous and ethanol extracts. Green tea extracts were four times less effective. Aqueous extracts showed 50% lower activity than equivalent ethanol extracts. Research proved that the antiradical activity of plant extracts is dependent on the mechanisms of oxidative activity of free radicals used and the chemical structure of antioxidants. The ethanoic extracts of green tea strongly inhibited the oxidation of canola oil compared to BHT. Oolong tea examined exhibited only moderate antioxidant activity.[61] Catechins were much more effective than theaflavins (TF) (TF1, TF2A, TF2B, and TF3) against the lipid oxidation of canola oil. Oxidation was conducted at 95°C by monitoring oxygen consumption and decreases in the linoleic and $\alpha$-linolenic acids of canola oil. Among theaflavins, TF3 was found to be the most effective.[62] The green and black teas showed much higher antioxidant activities against peroxyl radicals than 22 common vegetables.[63] The tea extracts (of rooibos, green, oolong, black teas) were strong inhibitors of $\beta$-carotene bleaching and active scavengers of DPPH radical.[64]

The antioxidant activity of catechins in oil systems was often contradictory. Matsuzaki and Hara[65] indicated that EGCG gave the strongest activity, whereas Tanizawa *et al.*[66] reported that EC showed the strongest antioxidative activity among catechins.

In a $\beta$-carotene-linoleate model system ECG possessed the strongest antioxidative activity and EGC showed the weakest effect.[67] The antioxidant activity of a

reconstituted catechin mixture in the crude extract was lower than that of the crude mixture itself.

The DPPH radical scavenging ability of catechins was EGCG > ECG> EGC> EC and that of theaflavins was TF2>TF1>TF.[68] EGCG, ECG and EGC showed higher lipid oxidation inhibition activity, as measured by the Rancimat assay, compared to BHT and theaflavins. The antioxidant activity of catechins and derivatives showed a marked difference depending on the substrate used for evaluation.[69] Green tea catechin performed like other hydrophilic antioxidants such as Trolox and ascorbic acid, which there have been shown to be active antioxidants in bulk oils, but these were prooxidants in the corresponding oil-in-water emulsions. These acetated and glycosylated catechin derivatives suggested, that the gallolyl moiety attached to flavan-3-ol at 3 position has a strong capability for scavenging the DPPH radical as well as the *o*-trihydroxyl group in the B ring, which elevates the radical scavenging effect above that of the *o*-dihydroxyl group.[70 -71] In the aqueous phase, the order of the effectiveness of catechins as radical ($ABTS^{\bullet+}$) scavengers was ECG> EGCG>EGC>EC>C. Against propagating lipid peroxyl radical species, EC and C were as effective as ECG and EGCG; the activity of EGC was the lowest.[72]In experiments of Terao *et al*.[73] EC and ECG retarded the accumulation of yolk phosphatidylcholine hydroperoxides when the suspension was exposed to water–soluble radical initiator (AAPH). The antioxidant activity of catechins against peroxyl radicals in a liposomal and aqueous system, except for EGC, was observed by Kondo *et al.*[74] Using LC/MS and spectroscopy studies these authors found that EC can be gently converted to an anthocyanin-like compound by radical oxidation.

### 1.5 The scope and aims of this research

In this work, the chemical composition of essential oils and extracts of fresh leaves and fermented leaves from Assam tea plantation in Chiang Rai province, Thailand were investigated. Furthermore, their antioxidant activities were evaluated. The results of this research obtained will be useful for food and beverage development and agricultural industry.

The aims of this research work can be summarized as follows:

1. To determine the chemical composition of volatile oils from fresh and fermented leaves of Assam tea by GC-MS

2. To investigate and quantify of phenolic compounds (GA, GC, C, EC, EGC, EGCG, ECG and GCG) and caffeine in fresh, steamed, fermented leaves (15, 30, 45, 60, 90, 120 and 150 days) extracts, steamed and fermented water (15, 30, 45, 60, 90, 120 and 150 days) by HPLC-UV

3. To evaluate the antioxidant activities (DPPH and FRAP) and total phenolic content of volatile oils and crude extracts from fresh, steamed, fermented leaves (15, 30, 45, 60, 90, 120 and 150 days), steamed and fermented water (15, 30, 45, 60, 90, 120 and 150 days)