# Antioxidative Activity, Polyphenolic Content and Anti-Glycation Effect of Some Thai Medicinal Plants Traditionally Used in Diabetic Patients

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**Abstract:** Ethanolic extracts of 30 Thai medicinal plants, traditionally used as alternative treatments in diabetes, were evaluated for antioxidative activity by the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method. They were evaluated *in vitro* for oxidative stress by thiobarbituric acid-reactive substance (TBARS) assay in pooled plasma of diabetic patients compared to without treatment of the extracts (control). The extracts were also assayed for protein glycation. The results showed that five plants had strong antioxidant activity: *Phyllanthus emblica* Linn. (PE), *Terminalia chebula* Retz. (TC), *Morinda citrifolia* Linn. (MC), *Kaempferia parviflora* Wall. (KP) and *Houttuynia cordata* Thunb.(HC), respectively. Thirty plant extracts were good correlation between total antioxidant activity and antiradical activity by TBARS as well as by glycation ($r = 0.856$, $p<0.01$ and $r = 0.810$, $p<0.01$). PE had stronger antioxidative activity as well as inhibition of TBARS and glycation than the other plants. The investigation showed that total polyphenol and tannin content of PE and the flavonoid content of HC were the highest. The results imply that these plants are potential sources of natural antioxidants which have free radical scavenging activity and might be used for reducing oxidative stress in diabetes.

**Key Words:** Thai medicinal plants, antioxidant activity, polyphenolic compounds, TBARS, glycation, diabetes

## INTRODUCTION

The etiology and pathophysiology of complications in diabetes mellitus (DM) such as coronary heart disease, nephropathy, retinopathy and neuropathy have been a topic of increased interest in recent years, particularly regarding the relationship between these complications and free radicals produced by hyperglycemia. Reports both *in vitro* and *in vivo* have implied that hyperglycemia causes an increase of free radicals in cells, which leads to acute toxicity and molecular destruction affecting the cellular mediators. Lipid peroxidation is produced by free radicals correlated with complication in DM such as coronary heart disease. Furthermore, glycation produced by hyperglycemia causes cell damage, which leads to diabetic complications [1]. There has been evidence that anti-oxidants, which can be found naturally or synthetically, can decrease or inhibit oxidation, implying that they could be used for reducing oxidative stress in diabetes [2-9].

There has been much research investigating the antioxidative ability and free radical scavenging ability of plant extracts [10-17]. In 1995, the WHO stated that of the 20,000 types of plant and herbs used, only 250 had been analyzed for their components and biochemical characteristics and, furthermore at least 25% of the active compounds found in drugs produced today are extracted from plants. Although the use of plants and herbs for treating disease and improving health has been around for many years, the study of their effectiveness in diabetes and its complications is less developed.

Reports on plant and vegetable research in Thailand reveal that high levels of antioxidants can be found, particularly, polyphenolic compounds [18-19]. Antioxidative ability and polyphenol properties protect against protein damage induced by iron and glucose [10]. The polyphenolic compounds like rutin, gallic acid, pyrogallol, catechin, quercetin and caffeic acid have been found in the plant infusions examined in Fig. (**1**) [20-23]. Village elders have passed down knowledge of the thousands of native Thai plants for many years from one generation to the next, but the basic scientific data about their effectiveness and mechanism of action are still lacking. The polyphenols-rich Thai medicinal plants would have antioxidative capacity and scavenge free radicals. This research intends to study the anti-oxidative ability of 30 Thai medicinal plants which have been used as an alternative treatment for diabetes. Ethanol crude extracts and their effects on oxidative stress by lipid peroxidation and protein glycation *in vitro* were studied. The plant extracts which demonstrated strong antioxidant activity will be used for HPLC analysis of polyphenolic compounds and total polyphenol, flavonoid and tannin contents.

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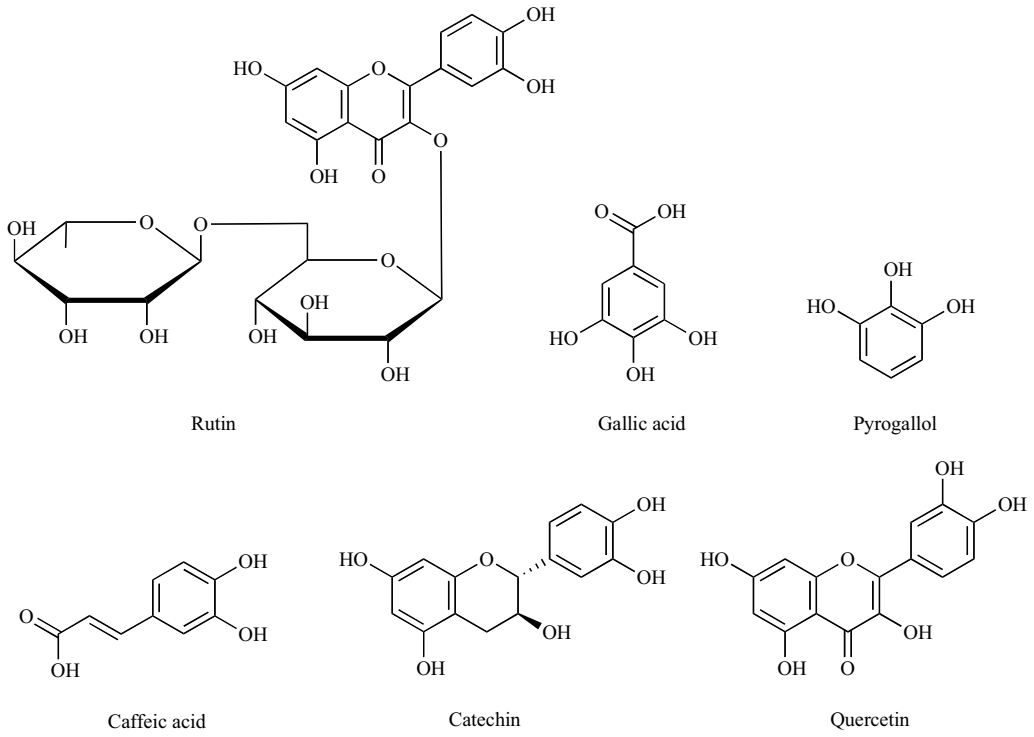


**Fig. (1).** Chemical structures of polyphenols.

## MATERIALS AND METHODS

### Chemicals

Malonaldehyde (MDA), Quercetin, Gallic acid, 2, 2'–azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2-Thiobarbituric acid (TBA) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Trolox was from Aldrich Chemical Company (Steinheim, Germany). Bovine serum albumin (BSA) and phenazine methosulphate (PMS) were procured from Fluka chemicals (Buchs Switzerland). The other chemicals were analytical grade.

### Preparation of Plant Extracts

Thirty fresh plants, traditionally used to treat DM were purchased from an organic farm in Chiang Mai, Thailand, during July 2006-February 2007. They were identified botanically from voucher specimens of the plants in the Herbarium of the Faculty of Pharmacy, Chiang Mai University, as shown in Table **1**. The plants were cleaned and cut into small pieces before being air-dried in the shade. The plants were homogenized using a blender and macerated in 95% ethanol (200 g of each plant/600 ml of 95% ethanol). The macerations were done at room temperature and protected from light for 3 days for three cycles. The extracts were filtered by filter paper, Whatman No.4, and evaporated under vacuum at 45 $^{o}$C until all extraction solvents were completely removed. The dry residues were obtained and the percent yield calculated. They were kept at 4 $^{o}$C, not exposed to light, for assay.

### Preparation and Identification of Plant Extracts Components by HPLC

The isolation of polyphenol from ethanolic plant extracts was performed by solid phase extraction (SPE) and HPLC. Briefly, 0.1 gram of each extract was dissolved with distilled water and 1-2 drops of HCl concentration were added and boiled 20 minutes. The SPE, C18 Cartridge column was washed with ethyl acetate 3 ml, methanol 3 ml and milli Q water 5 ml, followed by the deposition of the sample, 10 ml. Solvent was allowed to dry under reduced pressure and polyphenols, retained in the dissolved with methanol 1 ml. Preparation of standard solution using reference of standard compounds, such as rutin, gallic acid, pyrogallol, catechin and caffeic acid were dissolved in methanol.

The analytical HPLC system condition was Agilent 1100 Series diode-array detector high-performance liquid chromatograph. Polyphenolic compounds were identified with the diode array detector wavelengths at 200 nm and separation was done by ODS Hypersil (250 x 4 mm), 5µm, column oven was set up at 25 °C. Isocratic elution was employed with mobile phase consisting of water: 0.4% acetic acid: methanol: acetronitrile (70:20:5:5), flow rate of the mobile phase was 0.7 ml/min and the injection volume was 6 µl.

### ABTS Free Radicals Decolorization Assay

The ABTS radical-scavenging activity was analyzed according to the previous method [24]. This method is based on the ability of antioxidant molecules to quench the long-lived ABTS radical cation, a blue-green chromophore with characteristic absorption at 734 nm, compared with the Trolox, a water-soluble vitamin E analog. A stable stock solution of ABTS radical cation was produced by reacting 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate (final concentration) and allowed the mixture to stand in a dark at room temperature for 12-16 hour before use. The solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 units at 734 nm. Each plant extract

**Table 1. List of Scientific Name and Common Name from Parts of Medicinal Plants with Yield of Ethanolic Extract**

| Scientific Name | Common Name | Part Tested | Yield (%, *w*/*w*) |
|---|---|---|---|
| **Astringent** | | | |
| *Phyllanthus emblica Linn.* | Malacea tree | Fruit flesh | 2.58 |
| *Musa sapientum Linn.* | Banana | Flower | 4.83 |
| *Terminalia chebula Retz.* | Myrobalan wood | Fruit flesh | 8.83 |
| *Artocarpus heterophyllus Lamk.* | Jackfruit leaf | Leaf | 11.96 |
| **Bitter** | | | |
| *Morinda citrifolia* Linn. | Indian mulberry | Leaf and Fruit | 6.49 |
| *Gymnema inodorum* Decne. | Pukchiangda | Leaf and bud | 5.78 |
| *Sesbania grandiflora* (L.) Desv. | Scarlet wistaria tree | Flower | 5.83 |
| *Solanum torvum* Sw. | Devil's Fig | Fruit | 4.76 |
| *Kaempferia parviflora* Wall. | Krachai-Dam | Root | 5.39 |
| *Momordica charantia* Linn. | Balsam pear | Fruit | 10.96 |
| **Sour** | | | |
| *Lycopersicon esculentum* Mill. | Tomato | Fruit | 3.34 |
| *Houttuynia cordata* Thunb. | Heart leaf | Leaf | 1.93 |
| *Phyllanthus emblica* Linn. | Malacea tree | Fruit flesh | 2.58 |
| **Spicy** | | | |
| *Coriandrum sativum* Linn. | Ciriander seed | Seed | 5.15 |
| *Capsicum frutescens* Linn. | Chili pepper | Fruit | 5.45 |
| *Piper retrofractum* Vahl | Long pepper | Fruit | 4.83 |
| *Allium sativum* Linn. | Garlic | Seed | 9.25 |
| *Zingiber officinale* Linn. Adrak | Ginger | Root | 2.18 |
| *Costus speciosus* Koen. J.E. Smith | Crape ginger | Root | 0.24 |
| **Insipid** | | | |
| *Aegle marmelos* Correa | Beal | Leaf | 5.74 |
| *Coccinia grandis* (L.) Voigt. Syn. | Ivy Grourd | Leaf | 1.64 |
| *Eryngium foetidum* Linn. | Pukcheefarang | Leaf | 5.56 |
| *Ipomcea aquatica* Forsk. | Water morning glory | Leaf | 2.28 |
| *Acacia penneata* Linn. | Chaom | Leaf | 4.94 |
| *Coriandrum sativum* Linn. | Parslay | Leaf | 4.10 |
| *Piper samentosum* Roxb. | Betel | Leaf | 5.54 |
| *Apium graveolens* Linn. | Celery | Leaf | 4.35 |
| *Ocimum basilicum* Linn. | Hairy basil | Seed | 1.61 |
| *Pisum sativum* Linn. | Sugarpea | Pod | 7.36 |
| *Abelmoschus esculentus* Linn. | Okra | Pod | 4.83 |
| *Glycine max* Merr. | Soybean | Seed | 7.35 |

was dissolved in ethanol. An aliquot of each sample (20 µl) in ethanolic solution was added into 2.0 ml of ABTS free radical cation solution, the absorbance was monitored for 3 min at 734 nm, verified by a UV/VIS spectrophometer Jasco model 7800. The antioxidant activity of each sample was expressed as trolox equivalent antioxidant capacity (TEAC), defined as mg of trolox/g of dry weight of plant extract.

**Plasma Samples for Oxidative Stress in Diabetes**

Oxidative stress status, or presence of free radicals in diabetes, was measured by testing the pooled plasma samples collected from blood specimens from type II diabetics (fasting plasma blood glucose ≥ 200 mg/dl, 15 males and 15 females, aged over 35 years) from Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Thailand. Blood was collected in EDTA tubes and centrifuged at 3,000 rpm, 4 $^{o}$C for 15 min. Plasma was removed and kept frozen at -80 $^{o}$C for lipid peroxidation analysis. The Faculty of Medicine Ethics Committee for Human Research approved the protocol for blood collection (Reference No.0515 (05).17/212).

**Lipid Peroxidation Assay**

Thiobarbituric acid-reactive substances (TBARS) were used to evaluate lipid peroxidation [25] in modified blood plasma samples of diabetic patients. Briefly, plasma samples of type II diabetes patients were incubated with the extracts (1 µg/ml) in a shaking water bath under 95% $O_2$ / 5% $CO_2$ atmosphere at 37 $^{o}$C for 1 hour. After incubation, the sample was centrifuged at 14,000 rpm for 10 min. The supernatant portion was removed to measure for lipid peroxidation by TBARS assay. 40 µl of 0.2% butylated hydroxyl toluene was added to 375 µl of supernatant, the mixture was divided into three equal aliquots (one was used as a sample control and the others were used as duplicates). After 750 µl of phosphoric acid (0.44 M $H_3PO_4$) was added to each tube, 250 µl of 0.6% (w/v) thiobarbituric acid (TBA) reagent was added to both assay tubes and 250 µl of deionized water added to the control tube. All mixtures were incubated at 90 $^{o}$C for 30 min, then cooled at room temperature and the absorbance read at 540 nm. The data are expressed as malonaldehyde (MDA) equivalents (µM) using 1, 1, 3, 3-tetramethoxypropane as a reference standard.

**Glycation of Protein Analysis**

The procedure followed the method previously described [26]. Bovine serum albumin (BSA, 10 mg/ml), the extract, glucose (25 mM) and fructose (25 mM), were all mixed in 50 mM phosphate buffer, pH 7.4 (PBS), containing 0.02% (w/v) sodium azide. The 1.2 ml mixed solutions were incubated each with 0.3 ml BSA and the plant extracts at their final concentration (µg/ml). Glucose and fructose solutions were added to the reaction mixture at the end of the process for 30 min at room temperature (25 $^{o}$C). After incubating at 37 $^{o}$C in 5% $CO_2$ in air for 1 week, the fluorescence intensity (FI) was measured at an excitation wavelength of 350 nm and an emission wavelength of 450 nm by microplate reader (Backman coulter, model DTX 880 multimode detector). Results were expressed as percentage inhibition of the formation of glycated protein by the plant extracts, calculated according to the following formula: Inhibition of protein glycation (%) = 100 - [FI (sample) - FI (blank of sample)] x 100 / [FI (control) - FI (blank of control)], where FI (sample) is the FI in the presence of the sample extract in the mixture solution after incubated at 37 $^{o}$C , FI (blank of sample) is the FI of the sample extract in the mixture solution that was not incubated to check for error correction arising from unequal colour of sample extract, FI (control) is the FI of the mixture solution without the sample extract after incubation and FI (blank of control) is the same solution of the control that was not incubated. The percentage of inhibition of glycation was plotted against the sample extract concentration (µg/ml) to obtain the $IC_{50}$, defined as the concentration of sample extract necessary to reach 50% inhibition of the control, calculated from the linear regress equation.

**Determination of Total Polyphenolic Content**

Total polyphenolic content was determined by Folin-Ciocalteu reagent [27]. A dilute extract of each plant extract (0.5 ml of 1:10 g $ml^{-1}$) or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous $Na_2CO_3$ (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total polyphenolic content was determined by colorimetry at 765 nm. The standard curve was prepared using gallic acid in methanol: water (50:50, v/v) and expressed as gallic acid equivalent (mg GAE/g dry weight of plant extract).

**Determination of Flavonoid Content**

The flavonoids content was determined by aluminum chloride colorimetric method [28]. Each plant extract (0.5 ml of 1:10 g $ml^{-1}$) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance was measured at 415 nm. The standard curve was prepared by preparing various concentrations of quercetin in methanol and expressed as quercetin equivalent (mg QE/g dry weight of plant extract).

**Determination of Total Tannin Content**

The total tannin content in the lyophilized extracts was determined by modified previous method [29]. Each sample (0.1 ml) was mixed with 0.5 ml Folin-Denis reagent followed by 1 ml of $Na_2CO_3$ (0.5% v/v) solution and distilled water (up to 5 ml). The absorbance was measure at 775 nm within 30 min of the reaction against the reagent blank. Results were expressed as tannic acid equivalents (mg tannic acid/ g dried extract).

**Statistical Analysis**

Data were expressed as the mean±standard deviation (SD) of the duplicate samples which were replicated five times each. Pearson's correlation coefficient was used to test correlation between TEAC, inhibition of TBARS and glycation.

## RESULTS AND DISCUSSION

Thirty Thai medicinal plants which have been used as an alternative treatment in DM were obtained from various plant parts (Table **1**). They were extracted with 95% ethanol because it is the most widely-used solvent and safe to apply

in foods [30]. The plants were classified according to five taste groups: 1) astringent 2) bitter 3) sour 4) spicy 5) insipid. The extract yields of the five groups were 2.58-11.96, 4.76-10.96, 1.93-3.34, 0.24-9.25 and 1.61-7.36 %, respectively. The highest yield was from the *Artocarpus heterophyllus* Lamk. leaf from the astringent group (11.96%) The *Costus speciosus* Koen. J.E. Smith. root from the spicy group had the lowest yield (0.24%).

**Total Antioxidant Activity by ABTS**

*In vivo* condition, higher baseline levels of free radicals might not be specific for thalassemia but can be shared by other clinical situations such as diabetes. Over production of free radicals leads to oxidative stress causes cell damage, which leads to diabetic complications [1]. There has been evidence in iron-loaded diabetic patients with beta-thalassemia involves decreased antioxidants and free radicals-induced oxidative damage [31] and much research investigating the antioxidative ability and free radical scavenging ability of plant extracts [10-17]. Synthetic antioxidants have suspected low solubility and moderate antioxidant activity [32]. Hence, The anti-oxidants, which can be found naturally can decrease or inhibit oxidation, implying that they could be used for reducing oxidative stress in diabetes [2-9]. In this study, 95% ethanol crude extracts of 30 Thai medicinal plants were analyzed for their antioxidant activity, expressed as TEAC levels (mg Trolox/g dry weight of plant extract), as shown in Table **2**. The TEAC values of all samples ranged from 0.43 to 226.52 mg Trolox /g dry weight of plant extract. The five plant extracts with the highest level of antioxidant activity were *Phyllanthus emblica* Linn., *Terminalia chebula* Retz., *Morinda citrifolia* Linn., *Kaempferia parviflora* Wall. and *Houttuynia cordata* Thunb. (226.52, 95.02, 32.60, 21.21 and 18.93 mg Trolox /g dry weight of plant extract, respectively). Same as many studies have repor-ted phytochemicals with high levels of natural antioxidants acting as free radical scavengers [10-18, 28-29, 33]. Most plants from the astringent taste group had strong antioxidant activity, similarly reported in other study [34]. *Lycopersicon esculentum* Mill. from the sour taste group had the lowest level of antioxidant activity (0.43 mg Trolox /g dry weight of plant extract).

**Oxidative Stress Prevention using Lipid Peroxidation Assay**

The main mechanism of antioxidant action in food is radical scavenging, inhibiting the function of various membrane proteins such as lipid peroxidation or glycation *in vitro* [10]. In our model, oxidative stress was generated by blood plasma of diabetes patients and reaction of lipid peroxidation by TBARS. Plant extracts scavenged free radicals by their antioxidant activity. Table **2** shows the results of co-incubation with plant extracts at a concentration of 1 µg/ml. Among the 30 plants, *Phyllanthus emblica* Linn. had the highest antioxidant activity defined as free radical scavenging activity in lipid peroxidation.

This is significant, confirming the antioxidant mechanism, which explains the etiology and pathophysiology of the biological effects, especially in regards to cell damage and cellular degeneration, as seen in complications of DM [2, 35].

**Oxidative Stress Prevention using Glycation Assay**

Proteins (BSA) are modified by glucose creating advanced glycation end-products (AGEs) formation, which contribute to the complications in diabetes and other degenerative disorder diseases [36]. The free radicals have been shown to react in AGEs formation, which could be inhibited by using antioxidants, that is free radical scavengers. It is proposed that therapeutic strategies of anti-glycation are the use of alpha-oxoaldehyde scavengers, advanced glycation end-product receptor antagonists and inducers of enzymatic antiglycation defense [37]. In this experiment, we found the protective effect of all plant extracts on glycation as shown in Table **2**. The $IC_{50}$ value was determined based on scavenging activity per unit mass of inhibition of glycation in µg/ml. *Phyllanthus emblica* Linn. had the highest level of inhibition of protein glycation. As shown in Fig. (**2**), the result may be possible that rutin, gallate, pyrogallate and catechin compounds persisting in the extracts would compete with glucose to bind onto protein molecules, leading to inhibiting protein glycation.

**Correlation between Antioxidant Activity (TEAC) and Lipid Peroxidation and Protein Glycation**

There is evidence regarding the antioxidant potential of plants in their antiradical action [38]. In this study, the correlation between the antioxidant activity of 30 plants expressed as total TEAC values of the extracts and antiradical activity on oxidative stress expressed as inhibition of TBARS (Figure not show). The inhibition of TBARS (MDA; µM) was expressed as antiradical activity by converting their values to 1/MDA; µM ($r$ = 0.856, $p$<0.01) and the correlation between total TEAC values of 30 plant extracts and antiradical activity, as inhibition of protein glycation which was converted to demonstrate antiradical activity as $1/IC_{50}$ ($r$ = 0.810, $p$<0.01). The results showed that they correlated well, implying that the antioxidant activity of the 30 extracts play an important role in antiradical activity on lipid peroxidation and glycation. Similarly, the antioxidant components expressed different mechanisms of action, according to many plant studies reported, there is a correlation between total antioxidant activity and total polyphenolic compounds [10, 12, 18-19]. Particularly, we found that five plants with strong antioxidative ability had high correlation between total TEAC values and antiradical activity of TBARS ($r$ = 0.917, $p$<0.05). However, they correlated fairly in antiradical activity on glycation ($r$ = 0.722, $p$=0.168). Thus, their activity in reducing oxidative stress should be studied for active compounds, including total polyphenol, flavonoid and tannin content, along with the corresponding mechanism of action.

**Total Polyphenol, Flavonoid and Tannin Content of Five Plants with Strong Antioxidant Activity**

Polyphenolic compounds such as polyphenol, flavonoids and also tannins are very important plant constituents. Their antioxidant activity includes scavenging or chelating free radicals preventing lipid peroxidation and glycation [10, 20, 39-41]. In this study, we found different plant extracts contain different levels of total polyphenolic compounds (Table **3**). *Phyllanthus emblica* Linn. showed the highest total polyphenol and tannin content (2,536 mg GAE/g dry weight of plant extract and 461 mg TAE/g dry weight of plant extract)

**Table 2. Total Antioxidant Activity, Lipid Peroxidation Radical-Scavenging Activity and Glycation Inhibitory Activity of Medicinal Plants**

| Plants | TEAC (mg Trolox/g Crude Extract) | MDA (μM) | Anti-Glycation $IC_{50}$ (μg/ml) |
|---|---|---|---|
| **Astringent** | | | |
| *Phyllanthus emblica* Linn. | 226.52±0.02 | 0.07±0.01 | 0.01 |
| *Musa sapientum* Linn. | 6.52±0.00 | 1.34±0.01 | 3.63 |
| *Terminalia chebula* Retz. | 95.02±0.00 | 1.25±0.02 | 0.63 |
| *Artocarpus heterophyllus* Lamk. | 18.73±0.00 | 1.27±0.01 | 1.65 |
| **Bitter** | | | |
| *Morinda citrifolia* Linn. | 32.60±0.00 | 3.43±0.01 | 0.02 |
| *Gymnema inodorum* Decne. | 3.76±0.01 | 1.27±0.11 | 2.82 |
| *Sesbania grandiflora* (L.) Desv. | 1.93±0.01 | 2.69±0.03 | 1.75 |
| *Solanum torvum* Sw. | 3.68±0.00 | 0.30±0.00 | 3.48 |
| *Kaempferia parviflora* Wall. | 21.21±0.00 | 4.08±0.05 | 2.80 |
| *Momordica charantia Linn.* | 1.20±0.01 | 1.57±0.06 | 8.89 |
| **Sour** | | | |
| *Lycopersicon esculentum* | 0.43±0.00 | 0.22±0.01 | 3.30 |
| *Houttuynia cordata* Thunb. | 18.93±0.00 | 0.45±0.01 | 4.82 |
| *Phyllanthus emblica Linn.* | 226.52±0.02 | 0.07±0.01 | 0.01 |
| **Spicy** | | | |
| *Coriandrum sativum* Linn. | 2.23±0.00 | 1.34±0.03 | 3.06 |
| *Capsicum frutescens* Linn. | 3.71±0.02 | 5.30±0.02 | 2.61 |
| *Piper retrofractum* Vahl | 1.82±0.01 | 3.13±0.01 | 4.57 |
| *Allium sativum* Linn. | 0.72±0.00 | 2.69±0.00 | 4.87 |
| *Zingiber officinale* Linn. Adrak | 7.72±0.01 | 2.69±0.03 | 2.22 |
| *Costus speciosus* Koen. J.E. Smith | 2.02±0.01 | 1.27±0.08 | 3.98 |
| **Insipid** | | | |
| *Aegle marmelos* Correa | 4.67±0.02 | 1.12±0.03 | 3.21 |
| *Coccinia grandis* (L.) Voigt. Syn. | 9.02±0.04 | 2.84±0.04 | 2.23 |
| *Eryngium foetidum* Linn. | 5.94±0.02 | 2.01±0.07 | 3.29 |
| *Ipomcea aquatica* Forsk. | 4.67±0.00 | 5.07±0.06 | 2.88 |
| *Acacia penneata* Linn. | 2.46±0.00 | 4.78±0.07 | 7.72 |
| *Coriandrum sativum* Linn. | 2.23±0.04 | 6.87±0.05 | 4.91 |
| *Piper samentosum* Roxb. | 2.20±0.01 | 2.76±0.07 | 0.10 |
| *Apium graveolens* Linn. | 1.93±0.01 | 6.94±0.07 | 4.43 |
| *Ocimum basilicum* Linn. | 10.01±0.01 | 0.90±0.04 | 2.96 |
| *Pisum sativum* Linn. | 1.19±0.01 | 1.19±0.03 | 4.91 |
| *Abelmoschus esculentus* Linn. | 0.77±0.01 | 2.24±0.02 | 4.24 |
| *Glycine max* Merr. | 1.17±0.00 | 2.54±0.03 | 3.84 |

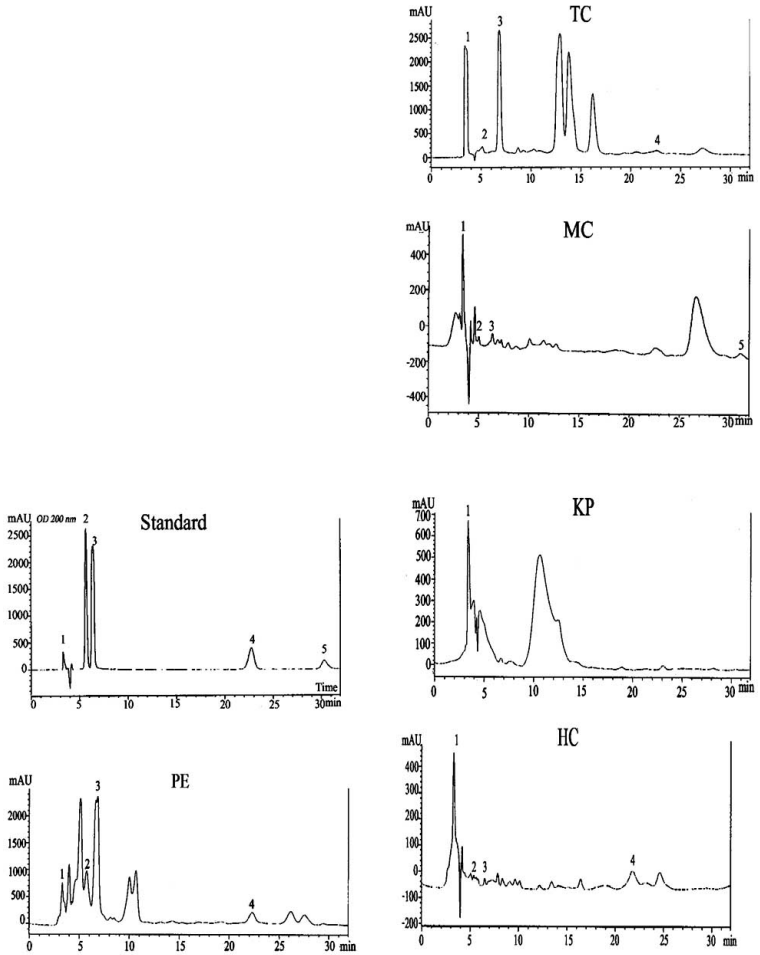


**Fig. (2).** HPLC analysis of polyphenols from standard and plant extracts; PE, TC, MC, KP and HC were detected at a wavelength of 200 nm, reference of standard compounds using rutin (1), gallic acid (2), pyrogallol (3), catechin (4) and caffeic acid (5).

**Table 3. Total Antioxidant Activity and Contents of Main Polyphenolic Compounds in Ethanol Extract of Five Plants**

| Plants | Antioxidant Activity (mg/g ) | Phenolic Acid (mg/g) | Flavonoid (mg/g) | Tannin (mg/g) |
|---|---|---|---|---|
| | Trolox Equivalent | Gallic Acid Equivalent | Quercetin Equivalent | Tannic Acid Equivalent |
| *Phyllanthus emblica* Linn. (PE) | 226.52±0.02 | 2,535.45±68.52 | 49.91±1.23 | 461.26±84.90 |
| *Terminalia chebula* Retz. (TC) | 95.02±0.00 | 2,275.45±436.35 | 99.21±5.99 | 330.86±55.30 |
| *Morinda citrifolia* Linn. (MC) | 32.60±0.00 | 49.60±7.78 | 68.06±0.43 | 44.24±4.61 |
| *Kaempferia parviflora* Wall. (KP) | 21.21±0.00 | 100.15±8.34 | 104.36±0.43 | 48.00±11.93 |
| *Houttuynia cordata* Thunb. (HC) | 18.93±0.00 | 295.20±22.49 | 191.17±1.71 | 96.40±14.56 |

Values expressed means±SD ($n$ = 5).

but the lowest total flavonoid content (50 mg QE/g of dry weight of plant extract) compared to other plants. *Houttuynia cordata* Thunb. had the highest flavonoid content (191 mg QE/g dry weight of plant extract). In addition, *Kaempferia parviflora* Wall. was high in total flavonoid content (104 mg QE/g dry weight of plant extract). Moreover, *Terminalia chebula* Retz. showed high levels of total polyphenol and tannin content (2,276 mg GAE/g dry weight of plant extract and 330 mg TAE /g dry weight of plant extract). However, *Morinda citrifolia* Linn. had the lowest total polyphenol and tannin content (50 mg GAE/g of dry weight of plant extract and 44 mg TAE /g dry weight of plant extract).

## HPLC Analysis of Polyphenolic Compounds

The polyphenolic compounds studied in this research are rutin (3.29 min), gallic acid (5.56 min), pyrogallol (6.35 min), catechin (22.72 min) and caffeic acid (30.24 min). HPLC chromatogram shows the major components of the plant extracts in Fig. (**2**). The major polyphenol in *Phyllanthus emblica* Linn., *Terminalia chebula* Retz. and *Hout-*

*tuynia cordata* Thunb.are rutin, gallic acid, pyrogallol and catechin. *Morinda citrifolia* Linn. consist of rutin, gallic acid, pyrogallol and caffeic acid. *Kaempferia parviflora* Wall. consists mainly the rutin.

## CONCLUSION

The antioxidant activity of 95% ethanolic extract of 30 Thai medicinal plants on diabetic oxidative stress was evaluated in different testing systems. The results show that *Phyllanthus emblica* Linn., contains the highest level of antioxidant activity, total phenolic and tannin content, antiglycation activity, and also lipid peroxidation scavenging activity. *Houttuynia cordata* Thunb. contains high levels of flavonoids. There was good correlation between total antioxidant activity and antiradical activity by TBARS as well as by glycation in 30 plant extracts. The present study suggests that these plants are a potential source of natural antioxidants such as rutin, gallic acid, pyrogallol, catechin and caffeic acid shown in the HPLC chromatogram. These compounds may play an important role in anti-oxidative stress properties by reducing the effect of oxidative stress in diabetes. The *in vivo* protective effect of these medicinal plants on diabetes should be further studied.

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