

Health Beneficial Effects and Chemical Profiles of Special Oolong Teas

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Abstract

Background: Tea blended with medicinal plants usually consists of a mixture of active compounds that can increase the beneficial health effects. Eternity tea is dried tea leaves blended with ginger (*Zingiber officinale*), lemongrass (*Cymbopogon citratus*), and celery (*Apium graveolens* L.), and peaceful rest tea is dry tea leaves blended with Indian gooseberry (*Phyllanthus emblica*), Turkey berry (*Solanum torvum* Swartz), and wild betel leaf bush leaves (*Piper sarmentosum* Roxb.). These teas have been widely used for their ability to reduce blood sugar levels. However, there are no previous reports on their pharmacological activities. This work investigates the chemical profiles and pharmacological activities, especially the antidiabetes activity, of special oolong teas blended with local Thai medicinal plants (peaceful rest tea and eternity tea).

Method: Eternity, Peaceful rest, and Oolong (*Camellia sinensis*) leaf samples were collected from Doi Chang organic tea plantation in Chiang Rai, Thailand. The chemical profiles of all tea samples were characterized by UHPLC-QTOF-MS. The extracts of these teas were investigated for their biological activities, including antioxidant, antiinflammatory, anticancer, and antidiabetic activities.

Results: Catechins were found in eternity and oolong teas. Peaceful rest tea contained catechins/dimeric catechins, flavonoid glycosides, and alkaloids. Biological testing showed that peaceful rest and eternity tea extracts inhibited nitric oxide formation in a dose-dependent manner with IC_{50} values of 42.6 and 48.9 $\mu\text{g/mL}$, respectively. Peaceful rest tea extracts inhibited the growth of K562 human erythroleukemia cells. Furthermore, peaceful rest tea with both ethanol and water extracts demonstrated higher α -glucosidase inhibition activities than acarbose ($IC_{50} = 75.71 \mu\text{g/mL}$), with IC_{50} values of 10.6 and 1.22 $\mu\text{g/mL}$, respectively.

Conclusions: Major antidiabetic compounds (5-*O*-Caffeoylquinic acid, kaempferol 3-*O*-glucosylrutinoside, and procyanidin B2) were found in peaceful rest tea. This finding corroborates that peaceful rest tea has greater antidiabetic effects than other teas against glucose and lipid levels. *In vivo* and clinical studies are therefore recommended to provide implications for the potential anti-diabetic properties of special oolong teas.

Background

Currently, tea consumption has become the most popular beverage in Southeast Asia [1-4]. Previous studies have reported that tea consumption might affect health benefits, including diabetes, cancer, cardiovascular diseases, obesity and central nervous system diseases [5]. In addition, the efficacy of tea has been revealed by its active ingredient, which depends on fermentation processing techniques [1].

Eternity, Peaceful rest and Oolong are part of the *Camellia sinensis* leaf family. Interestingly, tea blended with Thai medicinal plants' health might be increasingly efficient. Eternity and Peaceful rest tea are oolong products that blend local Thai medicinal plants with dry tea leaves through an invisible blending process, whereas Oolong tea is composed of organic tea leaves. The Eternity tea is dry tea leaves blended

with ginger (*Z. officinale*), lemongrass (*C. citratus*) and celery (*A. graveolens* L.). Peaceful rest tea is dry tea leaves blended with Indian gooseberry (*P. emblica*), Turkey berry (*S. torvum* Swartz.) and wild betel leaf bush leaves (*P. sarmentosum* Roxb.). Tea combined with medicinal plants usually consists of a mixture of active compounds that are frequently unidentified. *In vitro* bioactivity testing is required for regulatory strategies that can provide enhanced evidence of the safety, efficacy and quality of tea. Regarding this evidence, we are interested in the improved beneficial effects of oolong tea.

Therefore, the present study aims to investigate the characterization of chemical profiles in all teas by UHPLC-QTOF-MS. In addition, the crude extracts from these herbal teas will be investigated for their biological activities, including antidiabetic, antioxidant, anti-inflammatory, anticancer, anti-adipogenesis and cytotoxic activities.

Method

Plant material

Eternity tea (EN), Peaceful rest tea (PR) and Oolong tea (OL) leaf samples were purchased from Doi Chang Natural Tea Garden of Doi Chang Tea Co., Ltd., Mae Lao district, Chiang Rai, Thailand. All plant samples were authenticated by Charoensup R and compared with the herbarium specimen (QSBG No.27750) at Queen Sirikit Botanic Garden Herbarium. Voucher specimens of these samples were deposited at the herbarium of Medicinal Plants Innovation Center of Mae Fah Luang University with the references number of MPIC0136 (Oolong tea), MPIC0140 (Eternity tea) and MPIC0141 (Peaceful rest tea). The use of plant parts in present study compiles with the national guideline, procedures and conditions for permission to collect a native plants or forest plant species for breeding, study, experiment or research for commercial purposes.

Extraction of NR, PN and OL

The dried EN, PR and OL leaves were extracted with ethanol over three days at room temperature and evaporated under reduced pressure to provide the ethanolic extract. In addition, each tea sample was extracted with aqueous at 95°C for 30 min and freeze-dried to provide a water extract.

Ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) analysis

An Agilent 6500 Series LC Q-TOF System with a column (C-18 2.1*50 mm, 1.7 µm) from Zorbax Eclipse Plus was utilized. The mobile phase was composed of solvent A: H₂O + 0.1% formic acid and solvent B: ACN + 0.1% formic acid. The flow rate was 400 µL/min. The elution was performed starting at 5% B, which was linearly increased to 95% B in 1 min, after which a linear gradient was applied to 17% B in 13 min and 100% B in 22 min. The column was subsequently washed with 100% B for 2 min before returning to the starting conditions. The injection volume was 1.0 µL for the measurements in positive and negative mode. The instrument parameters were a gas temperature of 350 °C, gas flow of 13 L/min, and nebulizer

pressure of 45 psig. Agilent Mass Hunter Qualitative Analysis Software, version 8.00, was used for the initial processing of the LC/MS data. Compounds were revealed using the Molecular Feature Extractor Tool (MFE) tool in the software. CSV files were directly imported into Mass Hunter Profiler Professional (MPP), version 15.1, to generate the statistical analysis required to profile the samples such as HCA and PCA. One-way analysis of variance (ANOVA) was used to limit the set to those compounds that varied at the $P < 0.05$ level, and the sample set produced a list of thirty compounds that were used for statistical analysis.

Antioxidant activities

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The assay was measured using the modified method reported by Duangyod et al. [6]. Five hundred microlitres of tea extract solution was mixed with 500 μL of 59 μM DPPH solution in methanol. The mixtures were kept in the dark for 30 min, and the optical density was measured at 517 nm. (+)-Catechin hydrate was used as a positive control, and triplicate measurements were carried out. The percentage of scavenging activity was calculated by the formula given below:

$$\text{Scavenging activity (\%)} = \left(\frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}} \right) \times 100$$

where the concentrations of the extract required to scavenge 50% of DPPH scavenging activity under the assay conditions are defined as the IC_{50} .

2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay

The ABTS assay was based on the ability of different substances to scavenge 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid ($\text{ABTS}^{\bullet+}$) radical cations. The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.4 mM potassium persulfate (1/1, v/v) and leaving the mixture for 12 h in the dark. $\text{ABTS}^{\bullet+}$ was diluted with methanol to an absorbance of 0.706 ± 0.001 at 734 nm for measurement. The photometric assay was conducted on a mixture of 500 μL of ABTS solution and 500 μL of tea extract/control solution. After 7 min, the measurement was performed at 734 nm using a spectrophotometer. (+)-Catechin hydrate was used as a positive control, and triplicate measurements were carried out. The percentage of scavenging activity was calculated. The concentration of extract required to scavenge 50% of ABTS scavenging activity under the assay conditions is defined as the IC_{50} .

Total phenolic content

The total phenolic content was measured using a method reported by Duangyod et al. [6]. using the Folin–Ciocalteu reagent. Then, 800 μL of sample extracts and 200 μL of 15% Folin–Ciocalteu reagent were added to the test tube, and the volume was adjusted to 2.0 mL with water. The mixture was left for 5 min. Next, 1.0 mL of Na_2CO_3 (0.106 g/mL) was added. The mixture was kept in the dark at room temperature for 60 min. The absorbance was measured at 756 nm. The results were expressed as

micrograms of (+)-catechin hydrate equivalents per milligram of crude extract and micrograms of gallic acid equivalents (GAE) per milligram of crude extract.

α -Glucosidase inhibition assay

α -Glucosidase inhibition activity was performed under the following procedures. Fifty microlitres of tea extract/control was mixed with 100 μ L of α -glucosidase (0.35 U/mL) and incubated at 37 °C. After 10 min, 100 μ L of *p*-NPG (1.5 mM) was added into the mixture and incubated at 37 °C for 20 min. The reaction was terminated by the addition of 1,000 μ L of Na₂CO₃. Two hundred microlitres of the mixture was measured at 405 nm on a microplate reader by measuring the quantity of *p*-nitrophenol released from *p*-NPG. Acarbose was used as a positive control for the α -glucosidase inhibitor. The concentration of the extract required to inhibit 50% of α -glucosidase activity under the assay conditions was defined as the IC₅₀ value [1].

α -Amylase inhibition assay

The activity was measured using the method reported by Kusano et al., with slight modifications. Acarbose was used as a positive control. The substrate solution was prepared as follows: soluble starch (500 mg) was dissolved in 25 mL of 0.4 M NaOH and heated for 5 min at 100 °C. After cooling in water, the solution was adjusted to pH 7 with 2 M HCl, and DI water was added to adjust the volume to 100 mL. Tea extract solutions were prepared by dissolving each sample in 0.2 M acetate buffer (pH 7). Sixty microlitres of tea extract/control solution was mixed with 120 μ L of starch solution and incubated at 37 °C for 10 min. Then, 180 μ L of α -amylase (1 U/mL) was added to the solution and incubated for 30 min. The reaction was terminated by adding 240 μ L of 0.1 M HCl; then, 300 μ L of 0.1 mM iodine solution was added. After that, the solution was mixed with 500 μ L of 0.2 M acetate buffer, and the absorbance was measured at 412 nm using a spectrophotometer. Finally, the inhibitory activity (%) was calculated. The concentration of extract required to inhibit 50% of the α -amylase activity under the assay conditions is defined as the IC₅₀ value [7].

Glucose consumption

3T3-L1 preadipocyte cell lines were maintained in DMEM culture medium containing 10% FBS, 2 mM glutamine, 100 kU/L penicillin, 100 mg/L streptomycin, and a high glucose concentration (4.5 g/L) at 37 °C and 5% CO₂. Cells were detached from the culture flask with a solution of 0.25% trypsin and 1 mM EDTA. The trypsin digestion was stopped by PBS. The cells were washed twice and resuspended in low glucose (1.0 mg/mL) detection medium. The cell density was adjusted to a concentration of 1 × 10⁵ cells/mL, and cells were spread onto 96-well microtiter plates (100 μ L per well). The cells were cultured with serial samples at 37 °C and 5% CO₂ for 4–48 h. Insulin and metformin were used as positive controls in this experiment. At the end of incubation, 10 μ L of suspension or glucose standard medium (0–1,000 mg/L) was moved to another 96-well plate well by well. The glucose concentration remaining in the

suspension was measured by the glucose oxidase-peroxidase (GOD-POD) assay. Briefly, the reaction lasted 30 min at room temperature, and the absorbance at 510 nm was determined.

Glucose uptake

Cell culture and maintain

3T3-L1 preadipocyte and L6 myoblasts cells were obtained from American Type Culture Collection (ATCC, USA). All cells were incubated 37°C in a humidified incubator in an atmosphere with 5% CO₂

Cell differentiation

L6 myoblasts were grown in DMEM containing 10% (v/v) FBS, 1100 IU/L penicillin and 100 mg/L streptomycin in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C. Cells were reseeded in six-well plates or 24-well plates (for glucose uptake) at a density of 2×10⁴ cells/mL. After 48 h (~80% confluence), the medium was switched to DMEM with 2% (v/v) FBS and replaced after two, four, and six days of culture. Experiments were initiated on day seven when myotube differentiation was complete.

Glucose uptake assay

To determine the time response of L6 myotubes to samples, cells were incubated with samples or metformin for 24 h. Cells were washed with Krebs-ringer bicarbonate buffer twice. Cells were incubated with Krebs-ringer bicarbonate buffer for 1 h and starved in serum-free PBS containing 0.2% BSA for 1 h. After incubation, the cells were incubated with 2-NBDG for 20 minutes. The samples demonstrated fluorescence intensity at excitation/emission 485/530 nm.

Anti-adipogenesis assay

3T3-L1 preadipocyte cell lines were differentiated into adipocyte cells. The cells were treated with various concentrations of tea extracts and cultured in DMEM supplemented with 1 µM dexamethasone, 10 µg/mL insulin, and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) for 2 days. After 2 days, the medium was changed to DMEM containing 10 µg/mL insulin for 2 days. At the end of incubation, the medium was changed to DMEM until day 10. Lipid accumulation was assessed using oil red O staining. The samples were observed and recorded under a microscope. After that, the cells were dissolved in DMSO, and the absorbance was measured at 510 nm.

Cytotoxicity in RAW 264.7 cells using the MTT assay

Cell viability was measured by the MTT assay. RAW 264.7 cells were seeded at 4 × 10⁴ cells/well in 96-well plates and incubated at 37 °C and 5% CO₂ overnight. Cells were treated with different concentrations of tea extracts, including 6.25, 12.5, 25, 50, and 100 µg/mL, for 24 h. After 24 h, cells were washed with PBS and incubated with 0.5 mM MTT reagent for 4 h. The detection of formazan at 570 nm was

performed with an EZ read 400 microplate reader. The data were calculated as IC₅₀ values with GraphPad Prism 6.0 software.

Inflammation in RAW 264.7 cells using a nitric oxide (NO) assay

RAW 264.7 cells were seeded at 4×10^4 cells/well in 96-well plates and incubated at 37 °C and 5% CO₂ overnight. Cells were incubated with 1 µg/mL LPS for 1 h and treated with various concentrations of tea extract, including 6.25, 12.5, 25, 50 and 100 µg/mL, for 24 h. After 24 hours, 100 µL of Griess reagent was added to the samples for 10 min. Nitric oxide was measured at 570 nm with an EZ read 400 microplate reader. Additionally, the data are presented as the IC₅₀, which was calculated with GraphPad Prism 6.0 software.

Cancer cell growth inhibition assay

Anticancer activity was measured as the percentage of cell death using a resazurin assay. The K562 lymphoblastoid human erythroleukemia cell line was seeded at 4×10^4 cells/well in 96-well plates and treated with different concentrations of tea extract (12.5, 25, 50, 100, 250, and 500 µg/mL) for 24 h. Then, the cells were incubated with 0.15 mg/mL resazurin for 4 h. After 4 h, cell death at 600 nm was determined by a microplate reader. The results are reported as IC₅₀ values and were analysed by GraphPad Prism 6.0 software. In addition, the statistics were analysed to compare two means between the control and sample exposure groups. The statistics were analysed by one-way ANOVA with the Dunnett method using SPSS software.

Results

Liquid chromatography mass spectrometry analysis

The UHPLC-QTOF-MS data of NR, PN, and OL showed in Table S1-S3, their samples were acquired in positive ionization mode. The metabolite features were identified based on database searching and compared MS/MS spectra with literatures [5,8,9]. Comparison the UHPLC-LCMS profiles obtained from each teas, it was clear that PR extract have a difference metabolite with content of catechin dimer and flavonoids (Figure S1). These findings corroborate with their anti-diabetic activities. The ET and Oolong profiles were similar in the number of metabolite which can be attributed to the alkaloid and catechin derivative in both extract (Figure S2-S3 and Table S2-S3). Principal component analysis (PCA) was used to visualize the abundance variations for the 30 unidentified metabolites with significant differences across the three subtypes of oolong tea ($P < 0.05$) (Figure 1). In PCA score plot, each spot represents a sample (Figure 1A). The PCA analysis of oolong tea revealed that the samples can be divided to two groups: EN, PR and oolong. The PCA analysis data suggests that the catechin and flavonoid compounds in the PR may be most abundant components. EN and oolong samples can be assigned to one group, indicating that the catechin derivative compounds were highly similar in terms of types and content.

Effect of tea extracts on Antioxidant activities

The antioxidant activities of tea extracts were determined by the most common radical scavenging assays using ABTS and DPPH radicals. These assays are the most popular for assessing antioxidant-rich fruits, vegetables, and beverages in the US [10]. The results presented in Table 1 demonstrates that the antioxidant capacity detected by the DPPH assay was higher for all samples than that detected by the ABTS assay. The results reveal that all tea sample extracts have strong antioxidant activity.

Effect of tea extracts on Total phenolic content

All samples of tea extract were found to have more than 60 µg of gallic acid equivalents (GAE) per milligram of crude extract and more than 20 µg of (+)-catechin hydrate per milligram of crude extract (Table 1).

Effect of tea extracts on α-glucosidase and α-amylase inhibition assay

Aqueous extract of PR exerted the highest inhibitory activity against both enzymes compared to the acarbose, with IC₅₀ values of 1.22 µg/mL against α-glucosidase and less than 0.03 µg/mL against α-amylase (Table 2). Ethanol extract of PR exhibited less activity on α-amylase (IC₅₀ of 40.49 µg/mL), while a good activity was found on α-glucosidase (IC₅₀ value of 10.60 µg/mL) (Table 2). These results were related to a previous study that showed good α-glucosidase and α-amylase inhibition activities of black tea [1].

Effect of tea extracts on the glucose consumption of 3T3-L1 preadipocyte cells

Glucose consumption was used in insulin screening and was shown to increase after treatment with tea extracts. The results presented with PR aqueous extracts (50,100,250 and 500 µg/ml) showed the greatest enhancement of glucose consumption by 3T3-L1 preadipocyte cells in a dose-dependent manner compared with other tea extracts and metformin (Fig. 2).

Effect of tea extracts on Glucose uptake

All tea extracts stimulated glucose uptake in L6 myotube cells. Cells were incubated with each tea extract, and glucose uptake was measured with 2NBDG fluorescence. The results showed that all tea extracts at 100 µg/mL enhanced glucose uptake in L6 cells, especially the PR aqueous extract with 1.72±0.16 fold as compared to the control (Fig. 3).

Effect of tea extracts on intracellular lipid accumulation

3T3-L1 preadipocyte cells were differentiated into adipocytes. This experiment investigated the effect of tea water extracts on intracellular lipid accumulation in adipocyte cells. The cells were treated with 50 µg/mL tea water extracts for 10 days. Lipid droplets were stained with oil red O dye. The results revealed that all tea aqueous extracts reduced intracellular lipid accumulation in adipocyte cells, especially PR aqueous extract (Fig. 4A). Additionally, the PR aqueous extract showed the strongest reduction in intracellular lipid accumulation in adipocyte cells compared with the other tea extracts (Fig. 4B).

Cytotoxicity of tea extracts in RAW 264.7 cells using the MTT assay

RAW cells were investigated for viability using the MTT assay. The viability of cells was slightly decreased after treatment with tea extracts. Thus, the tea extracts (6.25-100 µg/ml) did not affect RAW cells. Regarding this evidence, the tea extract is non-toxic to normal cells, such as RAW cells (Fig. 5A and 5B).

Effect of tea extracts on inflammation in RAW 264.7 cells using nitric oxide (NO) inhibition assays

The inflammatory was detected nitric oxide secretion in RAW cells. The cells were investigated antiinflammatory with tea extracts (6.25, 12.5, 25, 50 and 100 µg/mL). The results showed that the extracts decreased nitric oxide in dose-dependent manner. In addition, PR aqueous extracts showed the lowest IC₅₀ with 43.82 µg/mL followed by EN and oolong aqueous extracts with IC₅₀ of 48.23 and 59.57 µg/mL respectively.

Effect of tea extracts on cancer cell growth inhibition

The IC₅₀ values of the eternity tea aqueous extract, peaceful rest tea aqueous and ethanolic extract were 490.91, 489.78, and 157.56 µg/mL, respectively. (Fig. 6).

Discussion

The results showed that the first principal component (PC1) accounted for 84.71% of the variability in the dataset, and the second PC (PC2) accounted for 4.44% of the variance in the data. PR showed negative loadings on PC1 and positive on PC2; however, EN showed positive loadings on PC1 and negative loadings PC2 and Oolong showed positive loadings on PC1 and PC2. The results indicate that their chemical profiles are different. Moreover, the PR extract showed interesting compounds: 1-Caffeoylquinic acid (t_R 15.24 min), Kaempferol 3-*O*-galactoside (t_R 19.54 min), and Procyanidin B2 (t_R 16.11 min); however, these compounds were not found in other teas (Fig. S1-S3 and Table S1-S3). These compounds stimulated antidiabetic and anticancer activity [11-14].

The tea samples showed high antioxidant activities compared to (+)-catechin hydrate (Table 1). This might be due to the phenolic content in those samples. The profound antioxidant activity of tea could be attributed to catechins and flavonoids. These results agree with previous reports that a greater amount of phenolic compounds leads to a more potent radical scavenging effect [1,6,15].

Table 1. Antioxidant activities and total phenolic content of tea extracts.

Sample	Antioxidation		Total phenolic content	
	IC ₅₀ (µg/mL)		(µg GAE/mg)	(µg (+)-catechin hydrate/mg)
	DPPH	ABTS		
PR (EtOH)	2.83 ± 0.01	3.35 ± 0.02	62.37 ± 0.0042	23.45 ± 0.0042
PR (aqueous)	1.84 ± 0.02	2.37 ± 0.02	62.07 ± 0.0012	23.32 ± 0.0012
EN (aqueous)	2.48 ± 0.01	3.12 ± 0.04	61.29 ± 0.0026	22.98 ± 0.0026
Oolong (aqueous)	2.77 ± 0.00	3.13 ± 0.00	68.37 ± 0.0021	26.02 ± 0.0021
(+)-catechin hydrate	0.82 ± 0.04	0.83 ± 0.01	-	-

α-Amylase and α-glucosidase are important enzymes involved in starch breakdown and intestinal glucose absorption, respectively. The inhibition of these enzymes can slow the passage of carbohydrates into the bloodstream, significantly decreasing the postprandial increase in blood glucose levels after a mixed carbohydrate diet and therefore can be an important strategy in the management of type 2 diabetes [16]. The PR extract showed high inhibitory activity on α-glucosidase and α-amylase (Table 2). The chemical profile of PR revealed the occurrence of alkaloids, catechins, and flavonoids (Table S3). The inhibitory activity of the both enzymes may be due, mainly, to the presence of catechins, and flavonoids [17-28].

Table 2. α-glucosidase and α-amylase inhibition assay.

Sample	α-glucosidase	α-amylase
	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)
PN (EtOH)	10.60 ± 0.44	40.49 ± 0.32
PN (aqueous)	1.22 ± 0.05	< 0.03
Acarbose	75.71 ± 0.34	28.73 ± 1.28

Additionally, procyanidin B2 and 5-*O*-caffeoylquinic were found in PR. Procyanidin B2 effect on a diabetic, that involved in glucose homeostasis [29]. Moreover, 5-*O*-caffeoylquinic regulated glucose transport which promote glucose plasma reduction thus 5-*O*-caffeoylquinic increased glucose translocation into cells [30]. Regarding in this evidence, both of these compounds might increase glucose consumption percentage in PR-treated-cells

In addition, 5-*O*-Caffeoylquinic acid also enhances glucose uptake in L6 Cells in dose and time dependent manner via AMPK activation that leads to GLUT4 and PPAR- γ stimulation. [31,32] Therefore PR was the strongest glucose uptake activity. Normally, patients with type 2 diabetes have impaired insulin-stimulated glucose uptake into cells; thus, patients with type 2 diabetes have a high level of blood sugar [33] The results suggest that PR might decrease blood glucose levels via glucose uptake stimulation (Fig. 3). The inhibitory activity of the glucose uptake may be due, mainly, to the presence of kaempferol and catechins derivatives [34-36]. Not only 5-*O*-Caffeoylquinic acid promote glucose uptake but also affect lipid metabolism. de Sotillo study reported fasting plasma cholesterol, triacylglycerol and liver triacylglycerols were reduced by 44%, 58% and 24% respectively in 5-*O*-caffeoylquinic acid-treated-rat [37]. 5-*O*-Caffeoylquinic acid upregulated PPAR- γ expression which plays a key role in glucolipid regulation [38]. Thus 5-*O*-Caffeoylquinic acid in PR might enhances lipid decreasing.

Inflammatory nitric oxide secretion was detected in RAW cells, and the anti-inflammatory effects of tea extract treatment were investigated. The results showed that both PR and EN water extracts decreased nitric oxide in a dose-dependent manner. In addition, the IC₅₀ values of the EN and PR water extracts were 48.87 and 42.56 $\mu\text{g}/\text{mL}$, respectively. The results suggest that EN and PR water had a strong anti-inflammatory activity. (Fig. 5B).

The results reveal that none of the tea extracts showed strong cancer cell growth inhibition activity against the K562 lymphoblastoid human erythroleukemia cell line (Fig. 6). However, the PR tea extracts showed better cancer cell growth inhibition activity than the Oolong extract. Studies have shown that PN tea contains procyanidin B2, a compound that inhibits cancer cell proliferation [39,40]. According to this previous study, PR tea presented strong cell growth inhibition.

Conclusion

Oolong tea is a popular beverage consumed daily worldwide. In the present study, we investigated the beneficial health effects and chemical profiles of several oolong teas. Peaceful rest and eternity tea were blended with Thai medicinal plants. Our results showed that all teas show anti-diabetic, antioxidant, anti-inflammatory, anti-cancer, and anti-adipogenesis effects in a safe manner. All of the tea sample extracts have strong antioxidant activity. The results related to previous studies showed that some catechins present in oolong tea or other polyphenols resulted in decreased free radical scavenging activity [41]. Another study reported that polyphenols not only induced antioxidants but also stimulated apoptosis in

cancer cells [42]. These anticancer effects were also found in our results, especially peaceful rest tea. PR contains procyanidin B2, which was not found in other teas. This compound inhibited cancer proliferation. Thus, it showed the highest cancer cell growth inhibition compared to other teas. In addition, Peaceful rest tea has high potential to inhibit α -glucosidase, α -amylase, glucose consumption, and glucose uptake. A previous study reported that quinolones increase insulin release from rat pancreatic islets via blockade of adenosine tri-phosphate (ATP)-sensitive potassium channels [1]. In addition, other previous studies have investigated the antidiabetic activity of gallic catechin in a rat model. A study reported that gallic catechin also stimulated an increase in insulin [5]. Regarding this evidence, Peaceful rest tea might enhance blood sugar reduction. Therefore, *in vivo* and clinical studies are therefore recommended to provide implications for the potential anti-diabetic properties of special oolong teas..

Abbreviations

2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), eternity tea (NR), gallic acid equivalents (GAE), glucose oxidase-peroxidase (GOD-POD), half maximal inhibitory (IC_{50}), 3-isobutyl-1-methylxanthine (IBMX), lipopolysaccharides (LPS), nitric oxide (NO), 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose (2-NBDG), mass hunter profiler professional (MPP), molecular feature extractor tool (MFE), one-way analysis of variance, (ANOVA), 4-nitrophenyl- β -D-glucopyranoside (*p*-NPG), Oolong tea (Oolong), peaceful rest tea (PR), Eternity tea (EN) principal component (PC), ultrahigh- performance liquid chromatography- quadrupole time- of- flight mass spectrometry (UHPLC-QTOF-MS)

Declarations

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Authors' contributions

Narawadee Rujanapun, Napaasawan Paojumroom, Thidarat Duangyod, Wuttichai Jaidee and Pravaree Phuneerub performed the experiments; Chuchawal Pringpuangkeo plant identification; Tharakorn Maneerat, Wuttichai Jaidee, Chakree Wattanasiri and Rawiwan Charoensup analyzed the data and drafted the manuscript and Rawiwan Charoensup revised the manuscript. All the authors have read and approved the final manuscript before submission.

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Availability of data and materials

The data associated with this study is available from corresponding author or the first authors upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Competing interests

The authors claimed no conflicts of interest.

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Figures

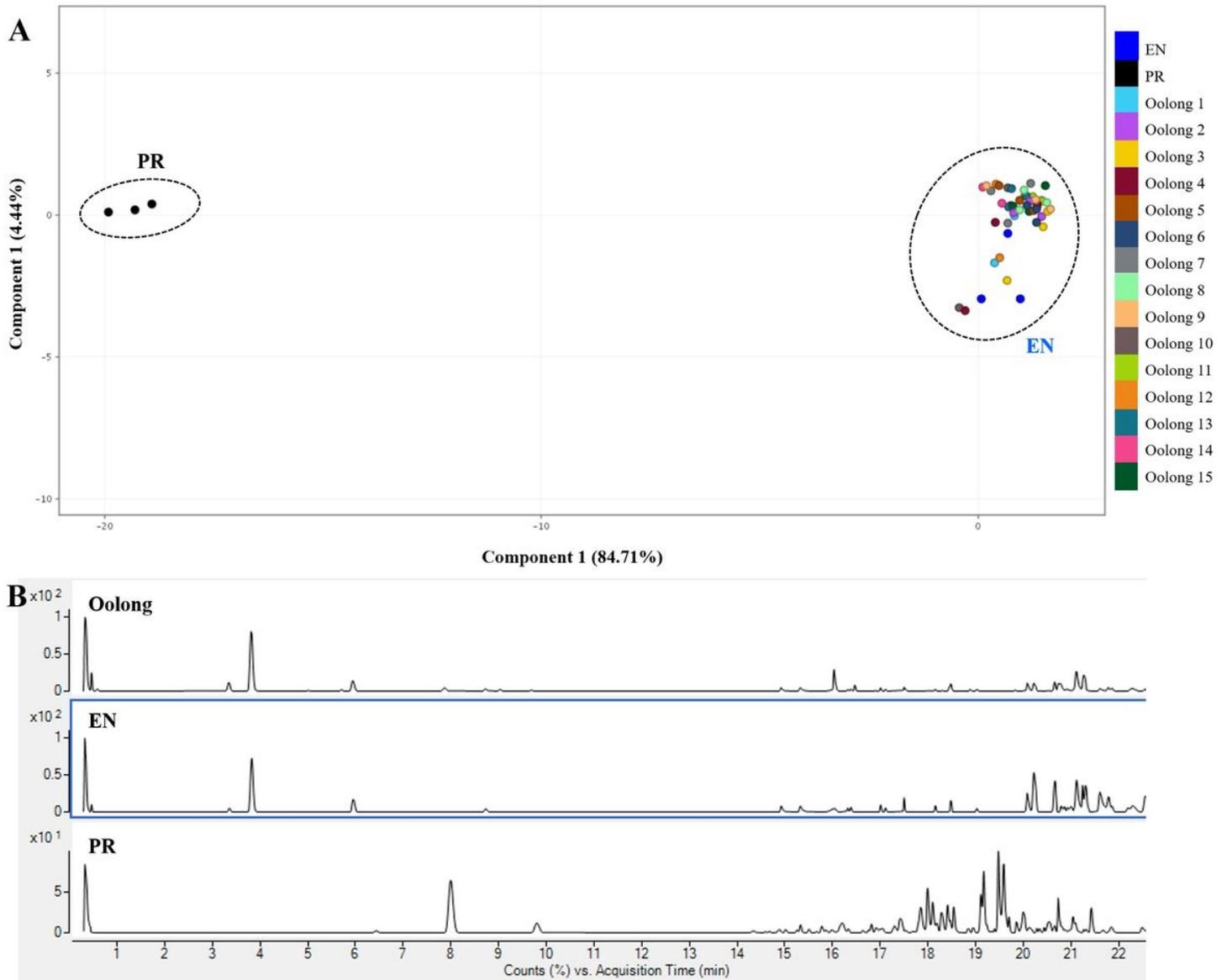


Figure 1

(A) Score plot PCA of the chemical compounds from EN, PR, and Oolong. (B) Total ion chromatogram of EN, PR, and Oolong extracts using UHPLC-QTOF-MS analysis.

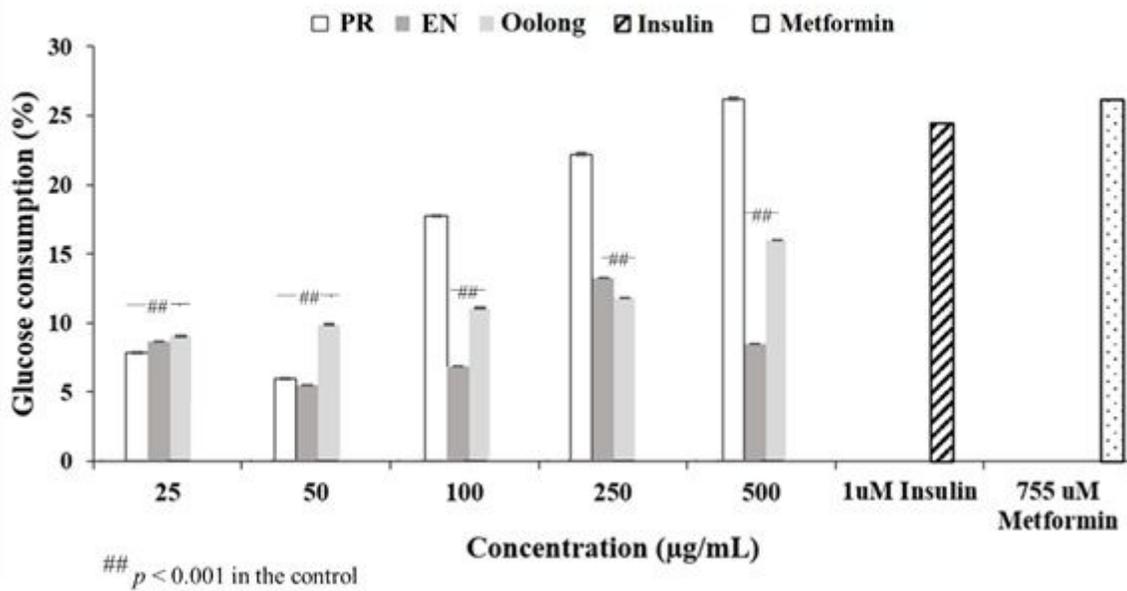
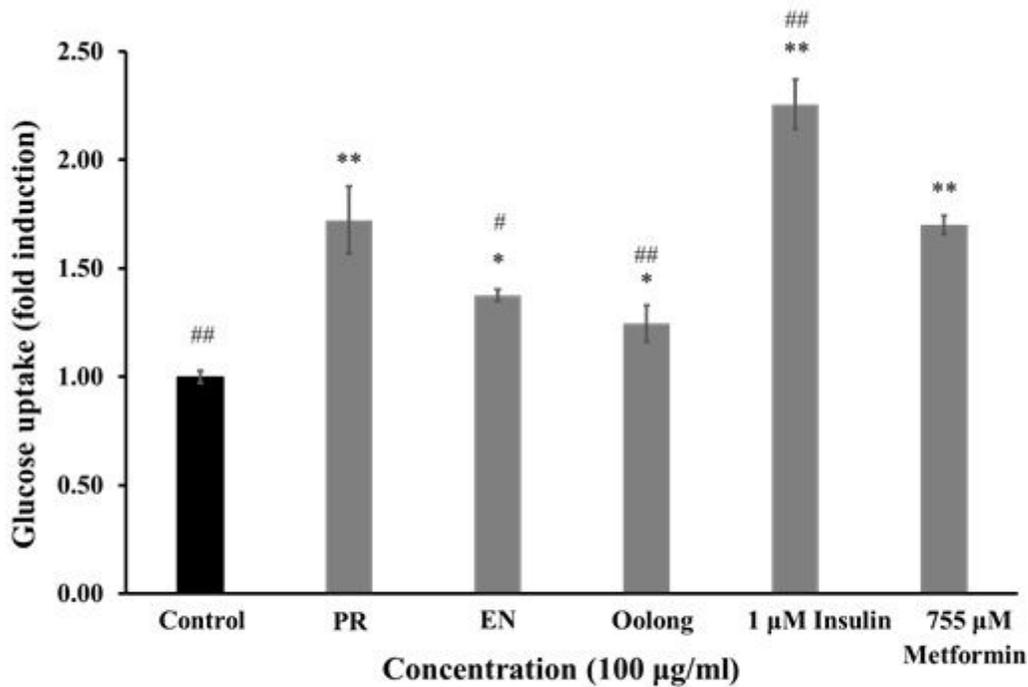


Figure 2

Effect of tea water extracts on glucose consumption in 3T3-L1 cells.



* $p < 0.05$, ** $p < 0.001$ in the control.

$p < 0.05$, ## $p < 0.001$ in the metformin.

Figure 3

Effect of tea water extracts on glucose uptake in L6 cells.

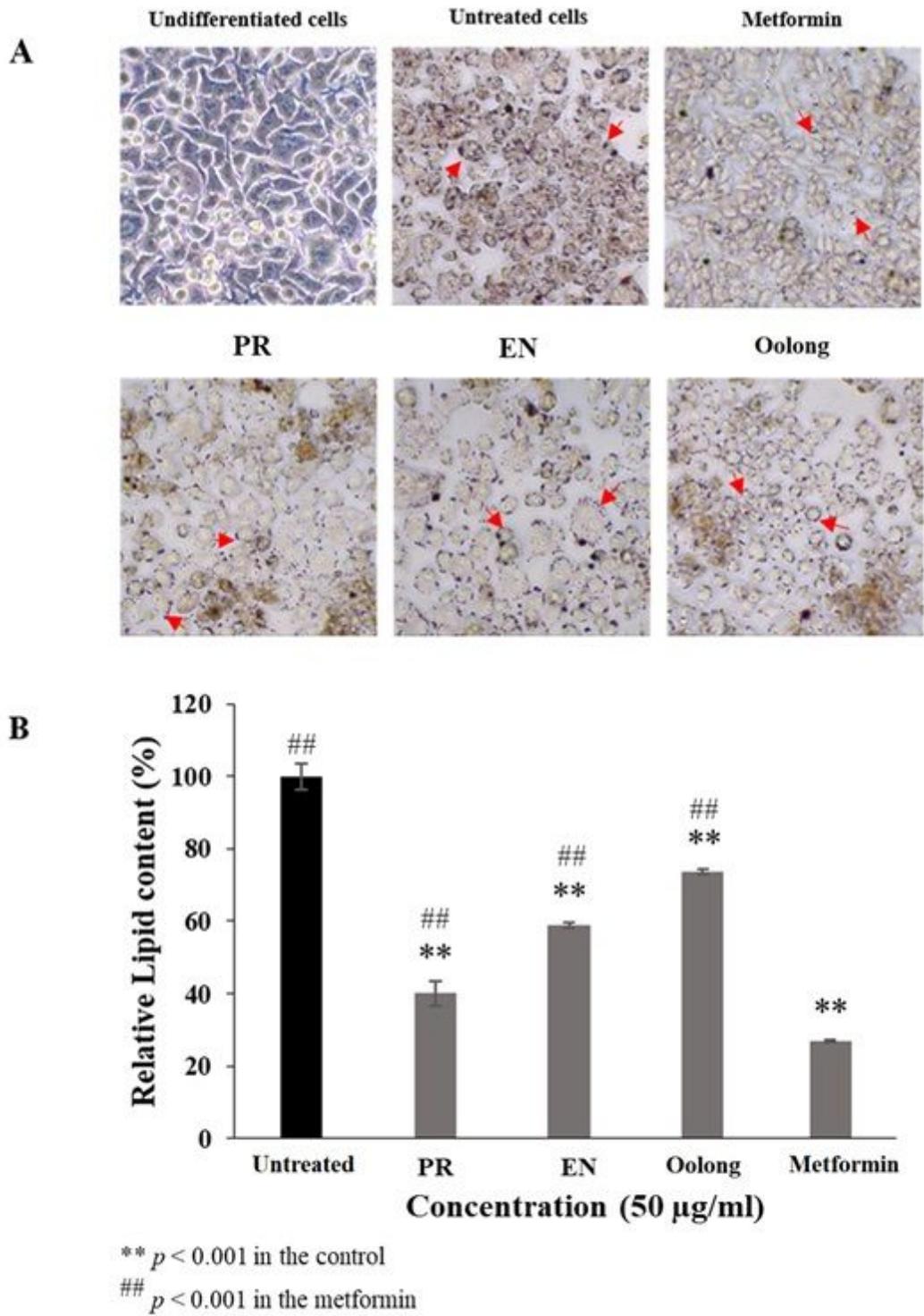


Figure 4

Effect of tea water extracts (50 µg/mL) on intracellular lipid accumulation. (A) Lipid droplets of adipocyte cells after red oil O staining. (B) Percentage of relative lipid content in adipocyte cells after tea water treatment. Arrows indicate lipid droplets.

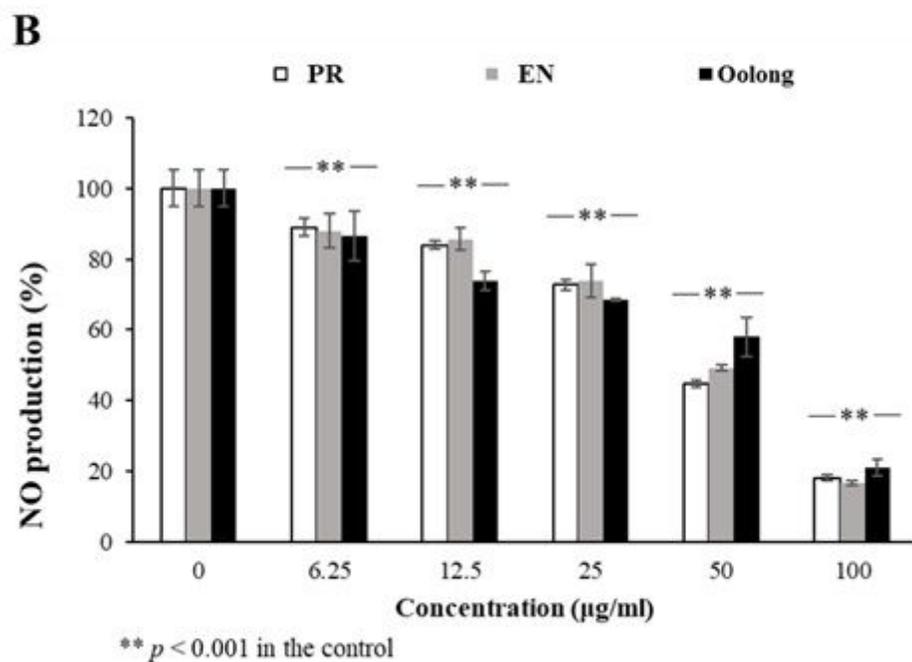
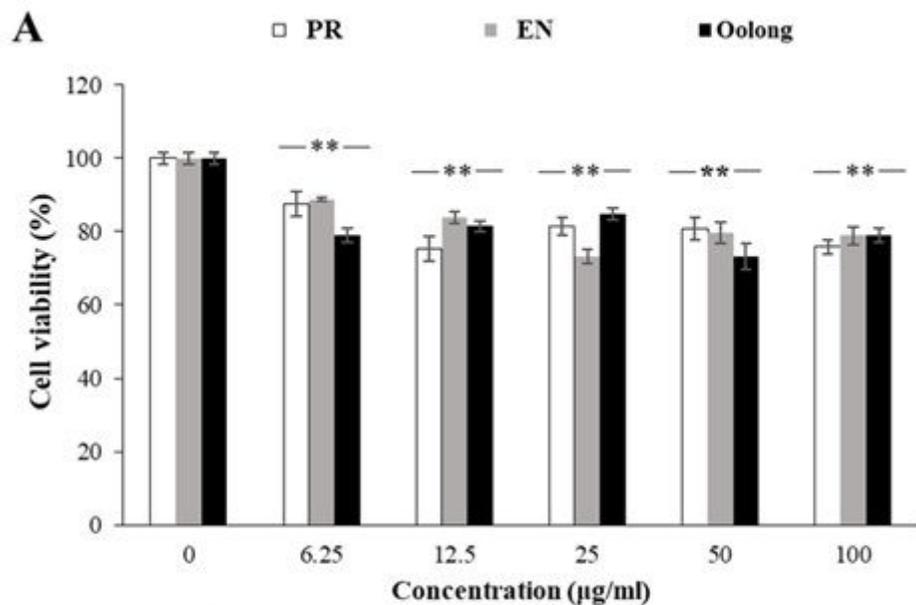


Figure 5

Effect of tea water extracts on RAW 264.7 cells. (A) Cell viability percentage. (B) Percentage of nitric oxide production in RAW cells after tea extract treatment.

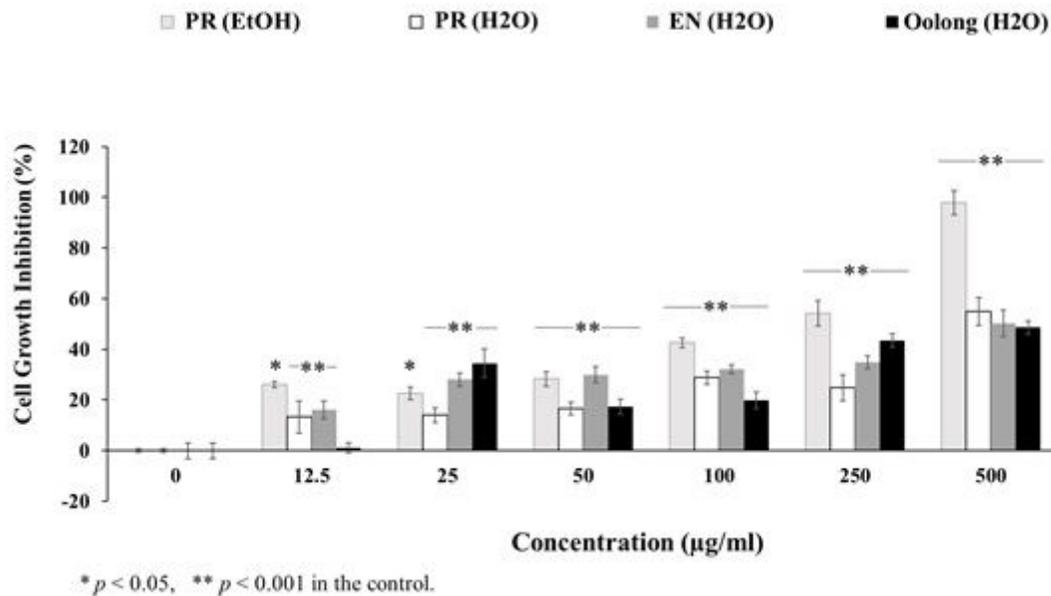


Figure 6

Cancer cell growth inhibition of K562 cells after tea extract treatment.

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