# *Alternanthera sessilis* and *Alternanthera bettzickiana* Improved Superoxide Dismutase and Catalase Activities in the Livers of Ovariectomized Mice

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**Abstract:** *Background:* Oxidative stress from reactive oxygen species can cause the damages to several cells or tissues. Ovariectomized (OVX) mice have been used as the oxidative stress model. *Alternanthera sessilis* (AS) and *Alternanthera bettzickiana* (AB), the Southeast Asia edible plants, have been traditionally used for promoting lactation, anti-inflammatory, wound healing, and antioxidation.

*Objective:* Impacts of AS and AB crude extracts on the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) including the levels of malondialdehyde (MDA) formation and glutathione (GSH) content were investigated in the livers of ovariectomized induced oxidative stress in mice to clarify their anti-oxidative potentials, compared to 17β-estradiol (E2).

*Materials and Methods:* Adult ICR mice were ovariectomized before treated with E2 (1 μg/kg/day, i.p.), or AS or AB (250, 500 mg/kg/day, p.o.), daily for eight weeks. The activity of CAT, SOD, and GPx and the levels of MDA and GSH in the mouse livers were determined.

*Results:* Ovariectomy significantly reduced the activities of hepatic CAT, SOD, and GPx enzymes while the level of MDA was increased. Extensive decrease of total GSH content, reduced GSH, and the ratio of GSH/GSSG in the OVX mice demonstrated occurrence of hepatic oxidative stress. E2, AS, and AB restored the CAT and SOD activities while that of GPx activity was not modified. The MDA level was extensively suppressed by E2, AS, and AB. The significant increase of the GSH/GSSG ratio was achieved as a result from the marked abatement of GSSG content by E2, AS, and AB, though the level of total GSH content and the reduced GSH was not accrued.

*Conclusion:* AS and AB possessed antioxidant benefit to improve the hepatic oxidant-antioxidant balance in the ovariectomized mice comparable to E2 did.

**Keywords:** *Alternanthera sessilis*, *Alternanthera bettzickiana*, catalase, glutathione, glutathione peroxidase, superoxide dismutase, malondialdehyde.

## INTRODUCTION

*Alternanthera sessilis* Linn. is an aquatic plant known by several common names, *i.e.*, sessile joy weed or dwarf copper leaf. *Alternanthera bettzickiana* Regel. is known as red calico plant. Both of *A. sessilis* and *A. bettzickiana* are the plants in Amaranthaceae family, used as edible vegetables in the Southeast of Asia [1-2]. In traditional medicine, *A. sessilis* and *A. bettzickiana* were used for relieve gastrointestinal distress, promoting lactation, prophylaxis of dementia, and nourishment [1]. Jalalpure *et al*. [3] revealed the wound healing activity and antimicrobial activity of the chloroform crude extract of *A. sessilis* were come from α-and β-spinasterol, β-sitosterol, and stigmasterol in this plant. Moreover, *A. sessilis* exhibited hepatoprotective effects against carbon tetrachloride, acetaminophen, or D(+)-galactosamine-induced liver injury in mice [4]. Wang & Zhang [5] found the effect of *A. bettzickiana* to lower body-temperature and the activity on calmodulin and $Ca^{2+}$-ATPase related anthocyanin accumulation in the plant seeds.

For anti-oxidation property, *A. sessilis* and *A. bettzickiana* inhibited free radicals from superoxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH) [2]. In addition, *A. sessilis* was reported anti-inflammatory effect in rats by inhibition of prostaglandin production [6]. Antioxidation and antithombolytic potentials of *A. sessilis* were related to its phenolic and flavonoids contents [7]. The acetone extract of *A. bettzickiana* showed the inhibitory effect on lipoxygenase activity [8].

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The free radical tissue-defense enzyme, such as catalase (CAT) and superoxide dismutase (SOD) are the important anti-oxidative mechanisms against reactive oxygen species (ROS) and particularly superoxide anion radicals in mammals [9-10]. However, the oxidative stress from imbalance between free radical species and the body's scavenging ability or antioxidant system can cause several damages to cells and organs [11]. Menopausal women or ovariectomized animals showed significant increase of oxidative stress with the depletion of antioxidant system such as an increase of malondialdehyde level (one of the end products in lipid peroxidation process), decreases of anti-oxidative enzymes including SOD, CAT, and GPx [12].

However, the data of *A. sessilis* and *A. bettzickiana* on oxidative stress in ovariectomized models was not yet completed. Therefore, in the present study we provided information on the effects of the ethanolic crude extract of *A. sessilis* and *A. bettzickiana* on activities of anti-oxidation enzymes namely CAT, SOD, and GPx, and lipid peroxidation in the livers of ovariectomized mice, compared to estradiol (E2). The results suggested the benefits of *A. sessilis* and *A. bettzickiana* on improvement of the oxidant-antioxidant balance for the use of these herbal plants as health supplements, at least in menopausal women.

## MATERIALS AND METHODS

### Materials

Estradiol (E2), gallic acid, quercetin, malondealdehyde (MDA), glutathione reductase, reduced glutathione (GSH), oxidized glutathione (GSSG), superoxide dismutase (SOD), xanthine, xanthine oxidase, nitroblue tetrazolium (NBT), ammonium molybdate, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), and 4-vinylpyridine were purchased from Sigma Chemical Co. (St. Louis, MO). All other laboratory chemicals were of the highest available purity from commercial supplier.

### Preparation of the Crude Extract

*A. sessilis* and *A. bettzickiana* were collected from Nonthaburi Province, Thailand in May 2012. After cleaning up, whole plants were chopped into small pieces and dried in hot air oven at 50°C. Then the dried plants were milled by grinder. One kilogram of each plant powder was refluxed three times with 80% ethanol at 50°C for 6 hours. The extract was evaporated and vaporized by a rotary evaporator and a freeze dryer, respectively. The yield of *A. sessilis* was 4.61 % of dry weight, and that of *A. bettzickiana* was 3.15 % of dry weight.

### Determination of the Total Phenolic and Flavonoid Contents

The total phenolic content of the crude extract of *A. sessilis* and *A. bettzickiana* were determined using the Folin–Ciocalteu method with some modifications [13]. The supernatant of *A. sessilis* and *A. bettzickiana* suspension was thoroughly mixed with the Folin–Ciocalteu reagent at a ratio of 1:10. After incubation for 3 min, 20% sodium carbonate was added. The mixture was gently mixed and allowed to stand for 30 min before being subjected to spectrophotometry at a wavelength of 750 nm. The total phenolic content was calculated as the weight unit equivalent to the dry weight of the standard gallic acid [14]. The aluminum chloride colorimetric method was applied with some modifications for determination of total flavonoid content. Briefly, the reaction mixture contained 10% aluminum chloride, 1 M sodium acetate, and a supernatant aliquot of the *A. sessilis* or *A. bettzickiana* suspension in a final volume of 200 ml. After incubation at room temperature for 30 min, absorbance of the reaction mixture was measured at a wavelength of 415 nm. Quercetin was employed as a representative standard of the total flavonoids content [14].

### Animals

Female ICR mice at 5 weeks of age were supplied by National Laboratory Animal Center, Mahidol University, Nakhonpathom, Thailand. All mice were housed in the Unit of Laboratory Animal Center, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand, and treated according to a research protocol approved by the Animal Ethics Committee for Use and Care of Khon Kaen University (Approval No. AEKKU42/2555). Mice were ovariectomized by the surgical removal of bilateral ovaries under the anesthesia with pentobarbital sodium (60 mg/kg, i.p.). An incision was made at cranial terminus 1 cm caudal to the 13$^{th}$ rib. The fur of dorsal surface is cleared away using blunt dissection and wetted by 70% ethanol. The connective tissue can be free from underlying muscle by sterile blunt dissection. The ovaries located in the fat under the dorsal muscle were removed using forceps and blunt dissection. The muscle incision was sutured by absorbable suture and the skin incision was closed by suture.

### Treatments

After ovariectomy (OVX) for three days, all treatments were started. The crude extracts of *A. sessilis* and *A. bettzickiana* were suspended in distilled water. E2 was dissolved in corn oil. Mice were divided into seven groups (n = 5). Group 1 was the sham operated control mice. Group 2 to 7 were ovariectomized mice (OVX). The control of OVX group was daily received only distilled water (OVX-NT). E2 at the dose of 1 µg/kg/day was daily intraperitoneally administered for eight weeks. The extracts of *A. sessilis* and *A. bettzickiana* (250, 500 mg/kg/day [15]) were daily intragastrically given for eight weeks. At 24 hours after the last treatment, the mouse livers were immediately excised and kept at -80°C for further assays.

### Determination of Catalase (CAT) Activity

The CAT activity was determined using the method of Chatuphonprasert *et al*. [16]. An aliquot of liver homogenate was incubated in 100 mM hydrogen peroxide substrate at 37°C for 1 min. The enzymatic reaction was terminated by addition of 16 mM ammonium molybdate followed by measurement of the yellow complex of molybdate and hydrogen peroxide by UV-Vis spectrophotometer at a wavelength of 405 nm. The percentage of inhibition of the yellow product forming was analyzed compared with the standard hepatic bovine CAT.

### Determination of Superoxide Dismutase (SOD) Activity

An aliquot of liver homogenate was extracted using chloroform and ethanol (3:5), and then centrifuged at 13,000×*g* at 4°C for 30 min for further analysis [16]. The supernatant was mixed with the reagent mixture (contained 1.2 mM xanthine, 140 mM ethylenediamine tetraacetic acid (EDTA), 140 mM NBT, 56 mM sodium carbonate, and 70 mg/ml BSA), followed by the xanthine oxidase solution (12.5 µU/ml). The reaction was incubated at 25°C for 20 min and stopped by 0.1 mM copper chloride before measurement of formazan production at a wavelength of 550 nm. The inhibitory percentage of formazan production was determined by compared with the standard bovine CuZn-SOD.

### Measurement of Reduced (GSH) and Oxidized (GSSG) Glutathione Contents

GSH and GSSG contents in the liver were determined using the glutathione assay kit, according to the manufacturer's instructions (Sigma, St Louis, MO, USA). Liver homogenate was extracted by 5% sulfosalicylic acid. After centrifugation at 10,000×*g* at 4°C for 10 min, the supernatant was collected and diluted for analysis of GSH and GSSG contents using DTNB. To measure GSSG content, the supernatant was pretreated with 4-vinylpyridine and incubated for 1 h at room temperature before performing the assay. The formation of thiol anions was measured at a wavelength of 405 nm at 1-min interval over a 5-min period. The GSH or GSSG content was determined as mmol/mg protein or nmol/mg protein, by comparison with the slope of the GSH or GSSG standard curve, respectively [16].

### Determination of Glutathione Peroxidase (GPx) Activity

The assay mixture sequentially contained 3.0 ml of a liver homogenate, 0.5 ml 0.25 M phosphate buffer, pH 7.4, 0.1 ml 25 mM EDTA, 0.1 ml 0.4 M sodium azide, 0.3 ml 50 mM GSH, and 0.1 ml 50 mM hydrogen peroxide. The reaction was terminated by adding 1.0 ml 5% sulfosalicylic acid at 10 s after the addition of the hydrogen peroxide. After centrifugation at 10,000×*g* at 4°C for 10 min, the supernatant was collected for measurement of GSSG as described elsewhere. The amount of GSSG contained in this reaction was accumulation of the GSSG created in this reaction, the endogenous GSSG in the sample, and the GSSG contained in the GSH solution. One unit of GPx activity was calculated from 1 mmol GSSG formed/min in the reaction at 30°C and pH 7.4 [16].

### Determination of Thiobarbituric Acid Reactive Substances (TBARS) Content

Lipid peroxidation was determined by measurement of TBARS formation as described previously [17]. In brief, a 500 µl aliquot of liver homogenate was incubated at 37°C for 1 h. Then, 1 ml of an equal volume of 40% (w/v) trichloroacetic acid (TCA) and 0.2% (w/v) thiobarbituric acid (TBA) was added. The reaction mixture was heated at 100°C for 15 min, before immediately cooled down. Then the reaction was stopped by 40% (w/v) TCA before subjected to centrifuge at 3,500 rpm at 4°C for 5 min. The TBARS formation in the supernatant was measured by using spectrofluorometry with an excitation wavelength of 528 nm and an emission wavelength of 551 nm compared with a standard of MDA.

### Statistical Analysis

The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test (SPSS ver. 17.0). Differences with $p<0.05$ was considered to be statistically significant.

## RESULTS

### Total Phenolic and Flavonoids Contents in the *A. sessilis* and *A. bettzickiana* Crude Extracts

The total phenolic content based on the equivalent amount of gallic acid in the ethanolic crude extract of *A. sessilis* and *A. bettzickiana* were 1.44±0.15 and 2.03±0.21 mg/g of dry weight, respectively. Using the standard curve generated by quercetin, the total flavonoid content of the ethanolic crude extract of *A. sessilis* and *A. bettzickiana* were 17.24±1.68 and 4.39±0.35 mg/g of dry weight, respectively.

### Improvement of CAT Activity by *A. sessilis* and *A. bettzickiana*

The activity of CAT enzyme was markedly decreased in the liver of ovariectomized mice (Figure **1**). Estradiol (E2) significantly increased the CAT activity, compared to the sham and the OVX-NT groups. The extract of *A. sessilis* at the dose 250 mg/kg/day for eight weeks restored the CAT activity to the sham level. Though the high dose of *A. sessilis* (500 mg/kg/day) increased the activity of CAT enzyme greater than the OVX-NT, the level was not comparable to that of the sham. Correspondingly, the extract of *A. bettzickiana* improved the hepatic CAT activity in the OVX mice, though the levels were not comparably restored to that of the sham level. These observations suggested that *A. sessilis* and *A. bettzickiana* possessed the ability to improve the CAT activity in the livers of ovariectomized mice.

### Improvement of SOD Activity by *A. sessilis* and *A. bettzickiana*

Not only the CAT activity was decreased in the livers of ovariectomized mice, but the activity of SOD enzyme was also depleted (Figure **2**). E2 significantly increased the SOD activity compared to the sham and the OVX-NT. Both of the *A. sessilis* and *A. bettzickiana* extracts increased the activity of SOD comparable to that did by E2. However, the dose-dependent effect of both extracts was not observed. These observations suggested that *A. sessilis* and *A. bettzickiana* had the potential to enhance the SOD activity in the livers of the ovariectomized mice.

### Effects of *A. sessilis* and *A. bettzickiana* on GPx Activity

The hepatic GPx activity was suppressed after ovaraiectomy (Figure **3**). There was no significant change of the GPx activity by either E2 or both extracts. Hence, these observations did not answer to the hepatoprotective effect of either the *A. sessilis* and *A. bettzickiana* extracts, or E2, in the livers of the ovariectomized mice.

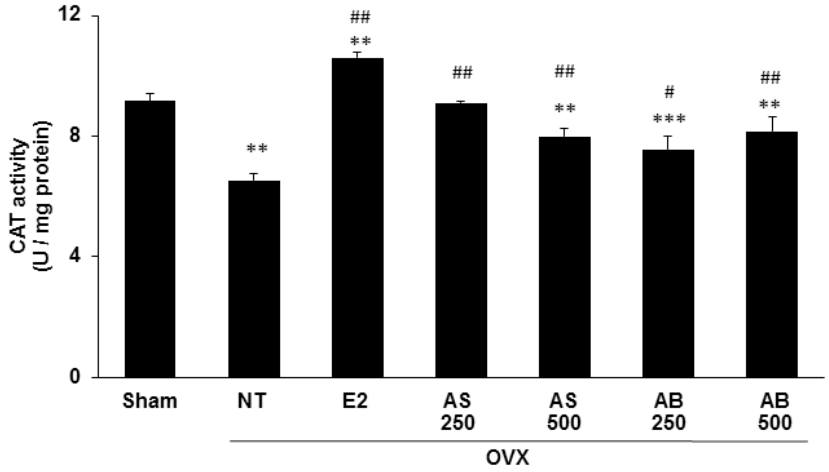


**Figure 1: Effect of *A. sessilis* and *A. bettzickiana* on CAT activity in the mouse livers.**

The ovariectomized mice (OVX) were daily given 17β-estradiol (E2, 1 μg/kg/day, i.p.), *A. sessilis* (AS, 250 or 500 mg/kg/day, p.o.), *A. bettzickiana* (AB, 250 or 500 mg/kg/day, p.o.), or distilled water (NT, 0.1 ml/mice) for 8 weeks. Mice were sacrificed 24 h after the last treatment and the livers were immediately excised for assessment of CAT enzyme activity. The data are presented as the mean±SD (n=5). A significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test. $^{*}p<0.01$, $^{**}p<0.001$ vs Sham; $^{\#}p<0.01$, $^{\#\#}p<0.001$ vs OVX-NT.

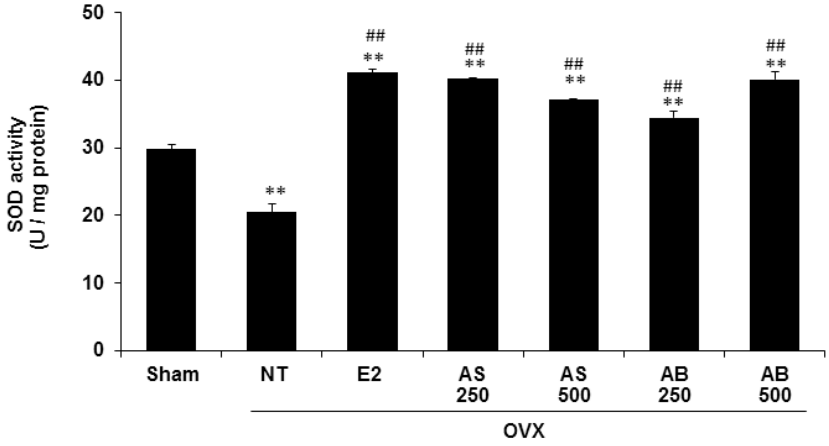


**Figure 2: Effect of *A. sessilis* and *A. bettzickiana* on SOD activity in the mouse livers.**

The ovariectomized mice (OVX) were daily given 17β-estradiol (E2, 1 μg/kg/day, i.p.), *A. sessilis* (AS, 250 or 500 mg/kg/day, p.o.), *A. bettzickiana* (AB, 250 or 500 mg/kg/day, p.o.), or distilled water (NT, 0.1 ml/mice) for 8 weeks. Mice were sacrificed 24 h after the last treatment and the livers were immediately excised for assessment of SOD enzyme activity. The data are presented as the mean±SD (n=5). A significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test. $^{**}p<0.001$ vs Sham; $^{\#\#}p<0.001$ vs OVX-NT.

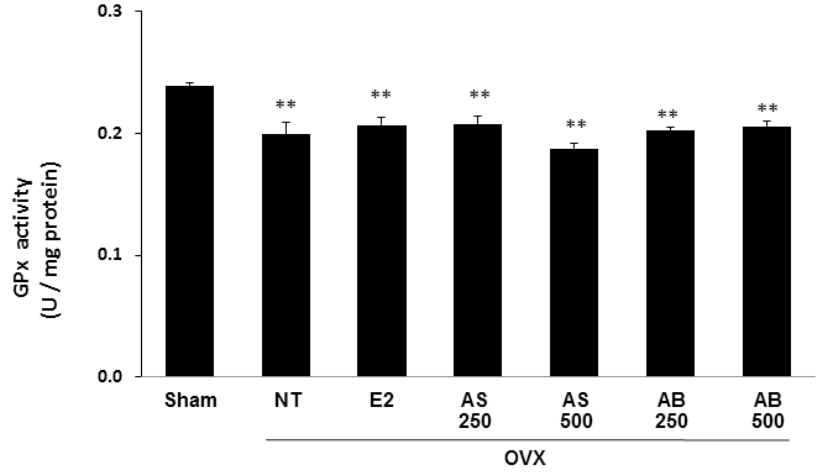


**Figure 3: Effect of *A. sessilis* and *A. bettzickiana* on GPx activity in the mouse livers.**

The ovariectomized mice (OVX) were daily given 17β-estradiol (E2, 1 μg/kg/day, i.p.), *A. sessilis* (AS, 250 or 500 mg/kg/day, p.o.), *A. bettzickiana* (AB, 250 or 500 mg/kg/day, p.o.), or distilled water (NT, 0.1 ml/mice) for 8 weeks. Mice were sacrificed 24 h after the last treatment and the livers were immediately excised for assessment of GPx enzyme activity. The data are presented as the mean±SD (n=5). A significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test. $^{**}p<0.001$ vs Sham.

### Improvement of Glutathione Content by *A. sessilis* and *A. bettzickiana*

All parameters of hepatic glutathione (GSH) content, namely the contents of total GSH, reduced GSH, and the ratio of GSH/GSSG, were significantly declined in the OVX mice, while the oxidized glutathione (GSSG) was markedly boosted in the livers of the OVX mice (Table **1**). These results indicated that the oxidative stress affected the GSH status in the OVX mouse livers. Neither the levels of total GSH content nor the reduced GSH was raised by E2 or the extracts of *A.* sessilis and *A. bettzickiana*. Interestingly, the GSSG content was significantly lowered by all treatments, except the high dose of the *A. bettzickiana* extract, comparable to that of the sham with the significant increase of the GSH/GSSG ratio by the extract of *A.* sessilis and *A. bettzickiana* at the dose of 250 mg/kg/day.

### Decrease of Lipid Peroxidation in the Mouse Livers by *A. sessilis* and *A. bettzickiana*

Formation of malondialdehyde (MDA) from the breakdown of polyunsaturated fatty acids serves as a

**Table 1: Effect of the Extracts of *A. sessilis* and *A. bettzickiana* on Total Glutathione, Reduced Glutathione, and Oxidized Glutathione Contents in the Mouse Livers**

| Treatment group | Total GSH content (nmol/mg protein) | Reduced GSH content (nmol/mg protein) | GSSG content (nmol/mg protein) | Ratio of GSH/GSSG |
|---|---|---|---|---|
| Sham | 50.26 ± 1.70 | 41.60 ± 0.34 | 8.04 ± 0.36 | 4.84 ± 0.22 |
| OVX-NT | 44.31 ± 0.47 * | 31.54 ± 0.79 * | 12.76 ± 0.79 * | 2.48 ± 0.22 * |
| OVX-E2 | 38.36 ± 2.94 *,## | 28.73 ± 0.17 *,## | 9.63 ± 0.17 ## | 2.98 ± 0.07 * |
| OVX-AS 250 | 34.41 ± 0.55 *,## | 26.10 ± 0.51 *,## | 8.31 ± 0.51 ## | 3.15 ± 0.25 *,# |
| OVX-AS 500 | 36.92 ± 0.85 *,## | 27.37 ± 0.23 *,## | 9.60 ± 0.23 ## | 2.85 ± 0.09 * |
| OVX-AB 250 | 41.33 ± 0.61 * | 31.61 ± 0.33 * | 9.65 ± 0.33 ## | 3.28 ± 0.15 *,## |
| OVX-AB 500 | 39.82 ± 0.36 *,## | 28.51 ± 0.95 *,## | 11.32 ± 0.95 * | 2.54 ± 0.28 * |

***Note.*** The data are presented as the mean±SD (n =5); Sham, sham operated mice; OVX, ovariectomized mice; NT, non-treatment; E2, estradiol benzoate (1 μg/kg/day, i.p., daily for 8 weeks); AS, *A. sessilis* extract (250 or 500 mg/kg/day, p.o., daily for 8 weeks); AB, *A. bettzickiana* extract (250 or 500 mg/kg/day, p.o., daily for 8 weeks). $^{*}p < 0.001$ vs Sham; $^{\#}p < 0.05$ ,$^{\#\#}p < 0.001$ vs OVX-NT.

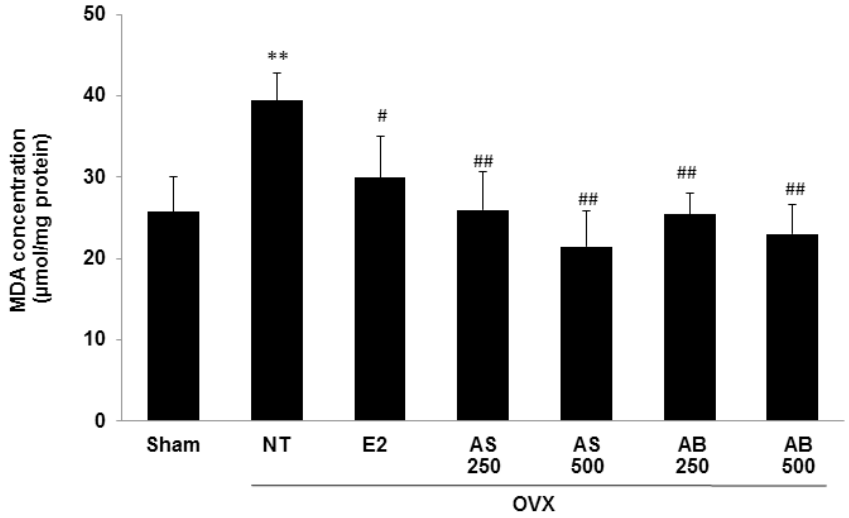


**Figure 4: Effect of *A. sessilis* and *A. bettzickiana* on lipid peroxidation in the mouse livers.**

The ovariectomized mice (OVX) were daily given 17β-estradiol (E2, 1 μg/kg/day, i.p.), *A. sessilis* (AS, 250 or 500 mg/kg/day, p.o.), *A. bettzickiana* (AB, 250 or 500 mg/kg/day, p.o.), or distilled water (NT, 0.1 ml/mice) for 8 weeks. Mice were sacrificed 24 h after the last treatment and the livers were immediately excised for measurement of MDA level. The data are presented as the mean±SD (n=5). A significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test. $^{**}p<0.001$ vs Sham; $^{\#}p <0.05$, $^{\#\#}p<0.01$ vs OVX-NT.

convenient index for determining the extent of lipid peroxidation reaction. The level of MDA was significantly elevated in the livers of the OVX-NT mice (Figure **4**). The treatments of E2, *A. sessilis*, and *A. bettzickiana* recovered the MDA formation in the livers comparable to the sham level.

## DISCUSSION

The level of estrogen hormone in ovariectomized mice is greatly dropped, leading to body weight gain, abdominal fat stores, increase of liver fat, and osteoporosis [18-19]. Therefore, ovariectomized mice were used as an animal model to reflect the pathological changes in perimenopausal and postmenopausal women [20-21]. Corresponded to our results, Muthusami *et al*. [22] revealed that the levels of lipid peroxidation and hydrogen peroxide were raised, and the enzymatic antioxidants like SOD and GPx were reduced in the ovariectomized albino rats when compared to the sham-operated control rats. Moreover, significant decreases of the GSH contents and the activities of CAT, SOD, and GPx enzymes in the livers and uteri of ovariectomized mice were recently reported [16], according to the present observations in the livers of the ovariectomized mice.

The prominent biological target of reactive oxygen species (ROS) is polyunsaturated fatty acid [12].

Mitochondrial defense mainly depends on SOD and GPx, while microsomal defensive system depends on CAT [12]. SOD removes superoxide anions (,O-2-•–.) by converting them to $H_2O_2$, which can be rapidly changed into water by CAT and GPx [23]. Moreover, GPx is one of the main enzymes involved in the glutathione redox cycle to convert hydroperoxide (ROOH) in combination with a change of reduced glutathione (GSH) into oxidized glutathione (GSSG) and to prevent the attack of polyunsaturated fatty acid by free radicals (RO• and •OH) derived from the unstable ROOH [12]. The present findings that both of *A. sessilis* and *A. bettzickiana* improved the activities of hepatic SOD and CAT enzymes noted the benefit of these evidences in the superoxide pathway. The GPx activity was not restored by the both extracts. The total glutathione content, the levels of GSH and GSSG, and the ratio of GSH/GSSG were not remarkably recovered by either *A. sessilis* or *A. bettzickiana*. Hence, both *A. sessilis* and *A. bettzickiana* might play the protective activity on the pathway of superoxide anion, but not on the hydroperoxide pathway. The dose dependent pattern of *A. sessilis* and *A. bettzickiana* was not observed on the investigated enzyme activities. Moreover, the dose of *A. sessilis* and *A. bettzickiana* at 500 mg/kg/day did not exhibit the greater anti-oxidative effect than the lower one (250 mg/kg/day). These observations indicated that *A. sessilis* and *A. bettzickiana* at the dose of 250 mg/kg/day for eight week were of optimal dose to improve the hepatic SOD and CAT activities in the ovariectomized mice.

Regarding the total flavonoid and total phenolic contents of the *A. sessilis* and *A. bettzickiana* extracts, correlation between these phytocontents and anti-oxidative effect was observed. Borah *et al*. [2] reported anti-oxidant activity of the *A. sessilis* extract isolated from different solvents, namely 90% methanol, 70% acetone, and 80% ethanol. The methanolic extract of *A. sessilis* showed the highest radical scavenging activity by DPPH, while ferrous chelating activity, superoxide radical scavenging activity, and nitric oxide radical scavenging activity of the acetone extract were better than the ethanol, and methanol extracts, respectively. Moreover, Borah *et al*. [2] reported the total phenolic content of *A. sessilis* was 1.40±0.07 mg/g of dry weight equivalent to gallic acid, which was comparable to our results. On the other hand, the flavonoid content of *A. sessilis* (0.37±0.01 mg/g of dry weight equivalent to rutin [2]) was not corresponded to our present observations (17.24±1.68 mg/g of dry weight equivalent to quercetin). The difference of flavonoid content might be due to the different flavonoid standards employed in these two experiments, in which the present study used quercetin as the flavonoid standard [13-14]. Only few data of *A. bettzickiana* was reported. Chen *et al*. [8] showed the inhibitory effect on lipoxygenase activity of the acetone extract of *A. bettzickiana*, corresponding with the anti-oxidation effect in the present study. Therefore, this is the first time to report the anti-oxidation property of *A. bettzickiana* in the livers of ovariectomized mice.

These findings supported the use of *A. sessilis* and *A. bettzickiana* as health supplements in the oxidative stress-induced condition, at least in menopausal women, according to their beneficent potentials to improve the levels of anti-oxidative stress enzymes, namely SOD and CAT, and to reduce the level of MDA formation in the livers of ovariectomized mice.

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