Yakae-Prajamduen-Jamod (ABP) recipe reduced anxiety behavior and brain oxidative damage in ovariectomy mice

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<td>Tantipongpiradet, Ariyawan; Faculty of Pharmaceutical Sciences, KhonKaen University, Pharmaceutical Chemistry Daodee, Supawadee; Faculty of Pharmaceutical Sciences, KhonKaen University, Pharmaceutical Chemistry Monthakantirat, Orawan; Faculty of Pharmaceutical Sciences, KhonKaen University, Pharmaceutical Chemistry Boonyarat, Chantana; Faculty of Pharmaceutical Sciences, KhonKaen University, Pharmaceutical Chemistry Matsumoto, Kinzo; Institute of Natural Medicine, University of Toyama, Medicinal Pharmacology Pitiporn, Supaporn; Chao Phya Abhaibhubejhr Hospital CHULIKHIT, Yaowared; Faculty of Pharmaceutical Sciences, KhonKaen University, Pharmaceutical Chemistry</td>
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Original Article

Yakae-Prajamduen-Jamod (ABP) recipe reduced anxiety behavior and brain oxidative damage in ovariectomy mice

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Abstract

Yakae-Prajamduen-Jamod (ABP) from Chao Phraya Abhaibhubejhr hospital is a traditional Thai herbal formula for treating perimenopausal and menopausal. However, its mechanism remains unknown. The present study aimed to investigate the effects of ABP on ovariectomy (OVX) induced anxiety and oxidative stress in mice. OVX mice exhibited not only anxiety in elevated plus maze and mirror chamber tests, but also brain oxidative stress when compared with control. OVX-induced behavioral and neurochemical alterations were attenuated by ABP treatment (500 mg/kg/day). We also analyzed antioxidant activities of ABP using DPPH and ABTS assays. ABP showed radical scavenging activity with IC₅₀ 58.59 and 44.99 µg/ml, respectively. Total
phenolic and flavonoid contents were 110.53±1.50 mg GAE and 152.25±0.5 mg rutin equivalents per gram extract, respectively. These findings suggest that ABP attenuated the OVX-induced anxiety and oxidative brain injury via antioxidative properties. Therefore, ABP is an alternative choice of anxiety disorder in the menopausal transition.

**Keywords:** ABP, menopause, ovariectomy, anxiety, lipid peroxidation,

1. Introduction

Recently, Thailand is the most rapid rates of ageing population among the developing country in the world (WHO, 2017). These reported is according to the increasing of menopausal woman in Thailand. The unstable of estrogen level during the menopausal transition is resulted to the unbalance of many systems in body such as central nervous system, cardiovascular system, endocrine system, immune system, reproductive system, renal system, and musculoskeletal system (Prossnitz & Barton, 2011). Particularly in central nervous system, estrogen is modulated various functions such as learning, memory, emotional, motivation, and sensory (Brinton, Yao, Yin, Mack, & Cadenas, 2015).

Normally, free radicals are generated in our body so the defensive mechanism against oxidation helps to protect the damage from over amount of free radical. Oxidative stress is defined as the imbalance of free radicals and antioxidant in defensive mechanism. The deprivation of estrogen can destroy the balancing of antioxidant system lead to over production of free radical and high oxidative stress (Al-Rahbi, Zakaria, Othman, Hassan, & Ahmad, 2014). Oxidative stress associated with neurological disease and aging by damaged to DNA, protein, and lipid. Evidence
suggests that the disruption of the central nervous system (CNS) areas results in various mental illnesses in menopausal women and an imbalance of the CNS redox state may influence such pathologies. Oxidative stress has been reported to involve in anxiety-related disorders (Hassan, Silva, Mohammadzai, da Rocha, & Landeira-Fernandez, 2014).

Yakae-Prajamduen-Jamod (ABP) recipe from Chao Phraya Abhaibhubejhr hospital is a traditional Thai herbal formula that is widely used for treating perimenopausal and menopausal symptoms in Thailand. ABP capsule is prescribed to patient by Thai traditional doctor in Chao Phraya Abhaibhubejhr hospital, Central public hospital in Prachinburi, Thailand. ABP consists of 22 medicinal herbs as follow; Cassia garrettiana Craib, Cassia siamea (Lam.) H.S.Irwin et Barneby, Derris scandens (Roxb.) Benth, Mesua ferrea L., Mammea siamensis Kosterm, Coriandrum sativum L., Myristica fragrans Houtt., Amomum testaceum Ridl., Cyperus rotundus Linn, Nigella sativa L., Piper ribesoides Wall., Aucklandia lappa DC., Artemisia annua L., Radix Angelica sinensis, Dracaena loureiri Gagnep, Tarenna hoaensis Pitard, Bridelia ovata Decne., Carthamus tinctorius L., Terminalia chebula Retz. var. chebula., Phyllanthus emblica L., Terminalia arjuna Roxb., and Aloe vera (L.) Burm.f. The medicinal efficacy of ABP may be due to the estrogenic activity and antioxidant activity of various herbs in the formula. However, the effect of ABP on ovariectomy induced anxiety and oxidative brain damage has not yet been reported. In addition, data indicate that OVX rats show behavioral alterations, including anxiety-like profiles associated with oxidative damage in different CNS areas (Hassan, Silva, Mohammadzai, da Rocha, & Landeira-Fernandez, 2014). Thus, the present study was aimed to investigate the
antioxidant property of this Thai herbal formula and explore the effects of ABP on
OVX mice by behavioral and oxidative parameter in different CNS areas of OVX mice.

2. Materials and Methods

2.1 Thai medicine formula for menopause

A traditional Thai medicine formula for menopause (ABP) was received from
Pho-Ngern-Abhaibhubejhr Osot, Chao Phraya Abhaibhubejhr hospital, Prachinburi,
Thailand, as a commercial product (ABP powder in capsule). It consists of twenty-two
dry medicinal herbs as described in table. 1.

2.2 The preparation of the ethanolic extract

ABP powder were taken out of capsule and extracted three times by maceration
methods with 95% ethanol at the room temperature. The ethanol extracts were
combined, filtered, and evaporated at 60 ºC. Two hundred grams of ABP powder
yielded 25.21 g of ethanolic extract. The extract was kept in -20°C along the experiment.

2.3 The measurement of antioxidant capacity

Sunrise™ microplate reader, TECAN was used for measuring the absorbance of
DPPH radical scavenging capacity and ABTS radical scavenging capacity. The
chemical reagent for DPPH were 1,1-diphenyl-2-picrylhydrazyl, trolox (Sigma-Aldrich,
MO, USA). The chemical reagents for ABTS were 2,2’-azino-bis-3-ethylbenzthiazoline-
6-sulphonic acid, diammonium chloride, potassium peroxodisulfate, trolox (Sigma-
Aldrich, MO, USA). All reagents used in this study were analytical grade. The DPPH
solution in ethanol was prepared and mixed with ABP ethanolic extract. The absorbance
was measured at 520 nm. The radical scavenging activity was calculated as a percentage
of DPPH discoloration (Kedare & Singh, 2011). The ABTS+• radical was prepared by
mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and
leaving the mixture for 4-16 h until the reaction was complete and the absorbance was stable. The ABTS++ solution was diluted with ethanol to an absorbance value of 0.7±0.02 at 734 nm for measurements. The photometric assay was conducted on 990 µl ABTS++ solution and 10 µl of ABP tested solution and incubate at room temperature for 15 minute. The absorbance was measured immediately at 734 nm. The antioxidative activity of the tested samples was calculated as the % inhibition of radical cation. IC₅₀ values were reported and compared with trolox, the reference antioxidant (Re et al., 1999).

2.4. Determination of total phenolic content (TPC)

The total phenolic content was determined by Folin–Ciocalteu’s colorimetric assay as modified by Todaro et al. (Todaro et al., 2017). Briefly, 20 µl of extract solution, 100 µl of 10% Folin–Ciocalteu reagent, and 80 µl of 7% Sodium carbonate (Sigma-Aldrich, MO, USA) were mixed and keep in room temperature for 30 minutes. The absorbance was measured at room temperature at 760 nm using a EnSight Multimode Plate Reader® (PerkinElmer, MA, USA). The total phenolic content was represented as mg of gallic acid equivalents (GAE) per gram of dry extract (mg GAE/g) using a standard curve with 0.1-1000 mg/L gallic acid (Sigma-Aldrich, MO, USA). All the determinations were performed in triplicates.

2.5 Determination of total flavonoid content (TFC)

Total flavonoid content of ABP ethanolic extract was determined by the aluminium chloride colorimetric method with slight modification (Woisky & Salatino, 1998). One mg of ethanolic extract was dissolved with 1 ml 80% ethanol. 20 µl of extract solution, 15 µl of 2.5% aluminium chloride, 20 µl of 10% acetate (Sigma-Aldrich, MO, USA) and 145 µl of distilled water were mixed and incubated at room
temperature for 15 minutes. The absorbance was measured at 430 nm using a EnSight Multimode Plate Reader® (PerkinElmer, MA, USA). Flavonoid content was expressed as rutin equivalents in mg per gram of dry extract using a standard curve with 0.1-1000 mg/L rutin (Sigma-Aldrich, MO, USA). All the determinations were performed in triplicates.

2.6 Experimental animals

Seventy-five female ICR mice (4-week old, 20-30g) were obtained from the National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand). Mice were housed on wood chip bedding in cages and giving food and water *ad libitum*. Housing conditions were 12-hour dark and light cycle (light 06.00 a.m. – 06.00 p.m.) under temperature control (22 ± 2 ºC) and constant humidity (45% ± 2%). All behavioral experiments were performed from 08.00 a.m. to 05.00 p.m. and each animal was used once. The experiment protocols was approved by Animal Ethics Committee of Khon Kaen University (ACUC-KKU-54/2559, Reference No. 0514.1.75/60).

2.7 Surgical operation and treatments

Ovariectomy (OVX) was conducted as previously described (Monthakantirat et al., 2014). The ovary and oviduct were removed from mice in OVX group. The sham group was intact-ovary mice which underwent operation. Three days after operation, OVX group were divided into four groups (n=10-15); (1) vehicle (0.5% sodium carbomethoxy cellulose, SPMC), (2) 1 µg/kg 17β-estradiol, (3) ABP 100 mg/kg, and (4) ABP 500 mg/kg. The sham group was received vehicle. 17β-estradiol (Sigma-Aldrich Co. LLC.) was suspended in corn oil. ABP was suspended in 0.5% SPMC as a vehicle. Vehicle, 17β-estradiol and ABP were orally administered once daily. During
07:00 a.m. – 09:00 a.m. for eight weeks. The behavioral tests were conducted one hour after treatment.

2.8 Behavioral tests

Elevated plus maze test (EPM)

The elevated plus maze test was used to evaluate anxiety-related behavior in rodent models with CNS disorders. EPM apparatus consists of two open arms and two closed arms (30x5cm) elevated to 35 cm-high from the floor, two closed arms with 15 cm-high black walls located in the opposite side (Figure 1). All treatments were performed one hour before placed on the intersection between arms (5x5 cm). Mouse was faced an opened arm to free exploration for 5 minute. The number of entries and the time spent in both closed and opened arms were recorded (Pellow, Chopin, File, & Briley, 1985). The %proportion of number of entries and time spend in each closed and opened arm were calculated using this equation,

The % proportion of time spent in opened arm = \frac{\text{The time spent in opened arm}}{\text{Total time spent}} \times 100

Mirror chamber test (MC)

MC is a test principle show approach-avoidance conflict behavior when faced with a mirror image (Toubas, Abla, Cao, Logan, & Seale, 1990). The mirror chamber apparatus consisted of two open-top connected boxes (35x35x30cm). The larger box contained wall with a mirror. The smaller box was lined with black wall without mirror (Figure 2). Mice were treated one hour before placed in the corner of the larger box with mirror side and allowed to free exploration for 5 minutes. The average time spent on dark sides was recorded (Lamberty, 1998).

2.9 The dissection of brain tissue
The animals were sacrificed by decapitation. Their hippocampus and frontal cortex were dissected out and kept at −80°C until use.

2.10 Determination of brain lipid peroxidation (TBARs assay)

Lipid peroxidation in brain (hippocampus and frontal cortex) was demonstrated according to Matsumoto et al. (Matsumoto et al., 1999) Hippocampus and frontal cortex were weighed and homogenized in 10 volumes in phosphate buffer (5 mM, pH 7.4). The homogenized brain was mixed with trichloroacetic acid and centrifuged at 8000 x g, 4 °C for 10 minutes. The supernatant was collected and incubated with 0.8% (w/v) 2-thiobarbituric acid at 100 °C for 15 minutes. The intensity of pink pigment formed from MDA-TBA condensation indicates the extent of lipid peroxidation. The complexes were determined by UV/visible spectrophotometer at 532 nm. The level of malondialdehyde (MDA) was determined as a standard. The protein contents in hippocampus homogenates were measured by Bradford method. (Chatuphonprasert et al., 2013) The results were represented as nmol of MDA/mg protein. (Grotto et al., 2009)

Statistical analysis

All data were expressed as the mean ± SEM. For multiple comparisons among different groups, data was analyzed by one-way analysis of variance (ANOVA) follow by the Tukey test. The significant difference was considered at p<0.05. SigmaStat® ver. 3.5 (SYSTAT Software Inc., Richmond, CA, USA) was used to analysis.

3. Results

3.1 The antioxidant capacity of ABP

The radical scavenging capacity of ABP was evaluate by DPPH and ABTS assay method. The value was expressed as IC$_{50}$ (The concentration of test compound
which inhibit 50% of the DPPH and ABTS radical). Trolox was used as standard antioxidant in this assay. The IC₅₀ value for radical scavenging capacity is represented in table 2A and 2B. The ethanolic extract of ABP in this study had IC₅₀ value for radical scavenging capacity in DPPH and ABTS assay were 58.59 and 44.99µg/ml, respectively.

3.2 The total phenolic and total flavonoid contents

Total phenolic content and total flavonoid content were determined from the calibration curves of gallic acid ((y = 2.5424x + 0.0977, R² = 0.9967)), and rutin (y = 0.9378x + 0.0042, R² = 0.9999), respectively. TPC was found to be 110.53±1.50 mg GAE/g extract. It is also observed that ABP extract are rich in flavonoids. The total flavonoid content was 152.25±0.5 mg rutin equivalents/g extract.

3.3 The effect of ABP on anxiety-like behavior in OVX model

EPM and MC were performed to evaluate the anxiolytic effect of ABP. EPM has closed arm which represent a safety zone for anxious mice. The result showed in figure 3 and 4. The elevated plus maze also revealed ovariectomy induced anxiety-like behavior in mice. The sham group spent significantly more percent proportion of entries in opened arm (p < 0.05) while the vehicle-treated OVX mice showed less percent proportion of entries in opened arm, indicating OVX-induced anxiety behavior. On the other hand, the OVX animals that received 17β-estradiol (1 µg/kg) and supplementation of ABP (500 mg/kg) for eight weeks exhibited significant improvement in the performance. There were statistically significant effects on estrogen deprivation in OVX mice on anxiety model (p < 0.001) by increasing of the percent proportion of time spent in closed arm. 17β-estradiol and ABP (500 mg/kg)-treated OVX groups showed
significantly reduction of time spent in dark compartment (p < 0.001) when compared to vehicle-treated ovariectomized group.

The anxiolytic effect of ABP was also conducted in MC test to confirm the anti-stress activity of ABP. The result of experiment was shown in figure 5. The sham-operated group spent significantly more time in dark compartment while the vehicle-treated control OVX showed less spending time in dark compartment, indicating ovariectomy induced anxiety behaviors. 17β-estradiol and ABP (100 and 500 mg/kg)-treated OVX groups showed significantly decrease in spending time in dark compartment (p < 0.001) when compared to vehicle-treated OVX group.

3.4 Brain lipid peroxidation (TBARs assay)

Lipid peroxidation is a source of free radical which causes the damages of lipid in brain (Lefevre et al., 1998). MDA, a secondary product of lipid peroxidation was estimated by measuring the TBARs levels and the results were exhibited in figure 6A-6B. There was significant effect of estrogen deprivation in OVX mice on lipid peroxidation in the brain (p<0.001) by increasing of MDA levels in hippocampus and frontal cortex when compare with sham control mice. OVX mice treated with 17β-estradiol- and ABP (500 mg/kg) showed a significantly decrease in the MDA levels in both brain areas when compare with OVX mice.

4. Discussion

The present study was aimed to investigate the effects of ABP on stress related-behaviors and oxidative stress in OVX mice model of menopause and clarify the possible mechanism. The amount of active constituents in ABP were also determined by HPLC method. Our results demonstrated that ovariectomy induced anxiety-like behavior as shown by EPM and MC tests. Moreover, OVX rats showed increased
TBARS levels in the frontal cortex and hippocampal areas. ABP treatment ameliorated anxiety behaviors and brain oxidative damage caused by ovariectomy.

It is well known that OVX model mimics many alterations observed in menopausal women, such as atrophy of uterus, cognitive dysfunctions, anxiety, oxidative stress, and strongly suggesting that estrogen deprivation is the important factor that makes women prone to menopause-related dysfunctions (Monthakantirat et al., 2014), (Chaves et al., 2009). In particular, depression and anxiety are among the main psychological symptoms related to sexual hormone deprivation in postmenopausal women (Chaves et al., 2009). These disorders emerge together due to a pro-oxidant status caused by estrogen deficiency, whereas estrogen itself can act as an antioxidant. The important part of estrogen for antioxidant effect is C-3 hydroxyl on phenolic A-ring (Prokai et al., 2003). Physiological circulating levels of estrogen in blood are associated with improved antioxidant status and also a lower incidence of mood disorders and oxidative stress-associated neurodegenerative disorders (Chakrabarti et al., 2014).

In the present work, the anxiety-like behavior was observed in OVX mice by approach-avoidance behavior when they were confronted to a mirror in MC test and increase proportion of time or entries in closed arm in EPM test. EPM, a pharmacologically validated assay of anxiety-like behaviors in rodents is based on the natural preference of rodents for enclosed versus exposed spaces. High percentage of time spent in the closed arms indicates the primary measure of anxiety-like behavior and is statistically decreased by administration of clinically effective anxiolytic compounds such as benzodiazepines (Rodgers & Dalvi, 1997). MC paradigm was based on the principle that many species show approach–avoidance conflict behavior when mirrors are placed in their environment. A distortion in the appearance of a
readily accessible environment via a compartment constructed of mirrored glass produces an anxiogenic state that was quantitatively/qualitatively. These are the non-invasive measure of anxiety-like behavior (Toubas et al., 1990). Treatment with ABP (500 mg/kg) showed significant attenuated anxiety-like behavior in both tests as well as estrogen replacement therapy (p < 0.001).

This study also examined the effect of ovariectomy induced oxidative brain damage. As a result, oxidative stress can alter neuronal function, neurotransmission, neurogenesis, and overall brain activity, and has also been implicated in anxiety disorders, depression, and high anxiety levels (Bouayed et al., 2009). The findings establish a link between oxidative stress and pathological anxiety. Many researchers have correlated oxidative stress and anxiety-like behavior. For example, ovariectomy causes oxidative stress in different CNS structures owing to depletion of antioxidant content leading to an anxiogenic profile (Behr et al., 2012). Da Silva Morrone and coworker observed elevated carbonylated protein levels in the hippocampus and striatum, increased TBARS levels in striatum and frontal cortex, and also a decrease in thiol content in striatal non-protein fractions, indicating a pro-oxidant effect in different brain structures after estrogen deprivation and these alterations related to the anxiety behaviors. ABP treated-OVX mice exhibited the reduction in TBARS level in the frontal cortex and hippocampus, suggesting a neuroprotective effect of ABP. Moreover, ABP extract also showed the potent radical scavenging activities in vitro by ABTS and DPPH assays.

In addition, it is also observed that ABP extracts are rich in phenolic and flavonoid compounds. There are many evidences reported that these chemical compounds possess the strong antioxidant activity. The phenolic compounds are very
important constituents in medicinal plants because of their scavenging ability due to their hydroxyl groups. The planar structure of flavonoids, number and position of their hydroxyl groups, as well as the presence of the C2-C3 double bond, are important for metal chelation, radical scavenging activity, and the inhibition of free radical producing enzymes (Badhani, Sharma, & Kakkar, 2015), (Azevedo et al., 2013), (Akinboro, Mohamed, Asmawi, Sulaiman, & Sofiman, 2011) and anxiolytic activity. On the other hand, phenolic compounds and flavonoids also exhibit the anxiolytic activity. Treatment of 10 mg/kg of gallic acid for 10 days significantly showed antianxiety-like activity in stress mice (Dhingra, Chhillar, & Gupta, 2012), and 300 mg/kg of rutin for 9 days exhibited the significant anxiolytic effect in rat (Hernandez-Leon, Gonzalez-Trujano, & Fernandez-Guasti, 2017).

Taken together, our findings suggest that OVX mice treated with ABP for 8 weeks showed decrease in anxiety-like behavior, and attenuation of oxidative stress in hippocampus and frontal cortex and the plausible mechanisms may be related to the active constituents in ABP such as gallic acid, and rutin which are strong antioxidants and anxiolytic agents. This is the first scientific-evidence that show the benefits of ABP oral administration against anxiety-like behavior and brain oxidative damage induced by hormone deprivation. Our results support the potential use of ABP as a natural alternative to HRTs.

5. Conclusions

In conclusion, a traditional Thai herbal formula for menopause (ABP) attenuated stress related anxiety disorder comparable to estrogen in OVX mice. This effect may associate to improvement of antioxidant activity against brain oxidative stress which was observed in hippocampus and frontal cortex. Therefore, ABP may offer an
alternative therapeutics choice of stress-related anxiety disorder observed in the menopausal transition.

Acknowledgments

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2011.122

Antioxidant activity applying an improved ABTS radical cation decolorization
00315-3


treatment on secondary metabolite content and antioxidant activity of poplar


Figure 1 Elevated plus maze (EPM) apparatus: (A) Side view, (B) Top view

Figure 2 Mirror chamber (MC) apparatus: (A) Side view, (B) Top view
Figure 3 The effects of ABP on OVX-induced anxiety-like behavior in the elevated plus maze test as the percent proportion of time. The value given in each column represents the mean ± S.E.M. (n = 10–12). Significant ANOVA effect were represent by * p < 0.05 vs. the ovariectomized group. # p < 0.001 vs. the sham-operated group.
Figure 4 The effects of ABP on OVX-induced anxiety-like behavior in the elevated plus maze test as the percent proportion of entries. The value given in each column represents the mean ± S.E.M. (n = 10–12). Significant ANOVA effect were represent by * p < 0.05 and ** p < 0.001 vs. the ovariectomized group. # p < 0.001 vs. the sham-operated group.
**Figure 5** The effects of ABP on OVX-induced anxiety-like behavior in the mirror chamber test. The value given in each column represents the mean ± S.E.M. (n = 10–12). Significant ANOVA effect were represented by * p < 0.05 and ** p < 0.001 vs. the ovariectomized group. # p < 0.001 vs. the sham-operated group.
Figure 6 The effects of ABP on lipid peroxidation in (A) hippocampus, (B) frontal cortex. Each column represents the mean ± S.E.M. (n = 3-5). # p < 0.01 vs. the sham-
operated group. ** p < 0.001 vs. the ovariectomized group. □ p < 0.05 vs. dose dependent (Cont.)
Table 1 Constituents of a traditional Thai medicine formula for menopause (ABP)

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<th>Thai name</th>
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</tr>
<tr>
<td>19</td>
<td><em>Terminalia chebula</em> Retz. var. chebula.</td>
<td>Sa-mor-thai fruits</td>
<td>1.5</td>
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<tr>
<td>20</td>
<td><em>Phyllanthus emblica</em> L.</td>
<td>Ma-kham-pom fruits</td>
<td>1.5</td>
<td></td>
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<tr>
<td>21</td>
<td><em>Terminalia arjuna</em> Roxb.</td>
<td>Sa-mor-ted fruits</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td><em>Aloe vera</em> (L.) Burm.f.</td>
<td>Yaa-dum resin</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 The radical scavenging capacity of a traditional Thai medicine formula for menopause (ABP).

(A) The radical scavenging capacity of ABP was evaluated by DPPH assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>% Inhibition (n=3) (mean ± S.E.M.)</th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trolox</td>
<td>10 µM</td>
<td>11.93±4.43</td>
<td>27.72 µM or 6.94 µg/ml</td>
</tr>
<tr>
<td></td>
<td>20 µM</td>
<td>30.22±13.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 µM</td>
<td>55.01±2.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 µM</td>
<td>80.42±1.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 µM</td>
<td>97.73±0.72</td>
<td></td>
</tr>
<tr>
<td>ABP</td>
<td>30 µg/ml</td>
<td>15.34±2.11</td>
<td>58.59 µg/ml</td>
</tr>
<tr>
<td></td>
<td>45 µg/ml</td>
<td>25.10±2.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 µg/ml</td>
<td>41.06±1.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75 µg/ml</td>
<td>55.17±2.68</td>
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</tr>
<tr>
<td></td>
<td>90 µg/ml</td>
<td>64.36±2.06</td>
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</table>
(B) The radical scavenging capacity of ABP was evaluated by ABTS assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>% Inhibition (n=4) (mean ± S.E.M.)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
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</thead>
<tbody>
<tr>
<td>Trolox</td>
<td>10 µM</td>
<td>9.71±0.42</td>
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</tr>
<tr>
<td></td>
<td>20 µM</td>
<td>24.56±1.59</td>
<td>40.78 µM or</td>
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<tr>
<td></td>
<td>30 µM</td>
<td>35.05±0.44</td>
<td>10.21 µg/ml</td>
</tr>
<tr>
<td></td>
<td>40 µM</td>
<td>49.35±0.42</td>
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<tr>
<td></td>
<td>50 µM</td>
<td>61.82±0.08</td>
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<tr>
<td>ABP</td>
<td>15 µg/ml</td>
<td>23.18±1.72</td>
<td>44.99 µg/ml</td>
</tr>
<tr>
<td></td>
<td>30 µg/ml</td>
<td>37.34±0.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45 µg/ml</td>
<td>52.10±1.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 µg/ml</td>
<td>63.97±1.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75 µg/ml</td>
<td>73.46±2.03</td>
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</tbody>
</table>