

**Effects of *Aia tempayang* on total antioxidant capacity and malondialdehyde level against 7,12-dimethylbenz(a)anthracene-induced breast cancer in rats**

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**Abstract**

Breast cancer occurs due to the abnormal growth and proliferation of cells derived from breast tissue that is caused by continuous stimulation by carcinogenic factors. Breast cancer can be prevented by consuming food that contains antioxidants, such as *Aia tempayang*. *Aia tempayang* is a traditional drink originating from West Sumatra. It is made from sappan wood, *pangdahai*, and basil seeds. Each of its ingredients contains compounds such as antioxidants that can help prevent breast cancer. This study aimed to prove the effectiveness of the administration of *Aia tempayang* in preventing breast cancer in rats induced by 7,12-dimethylbenz(a)anthracene (DMBA). Healthy female Sprague Dawley rats (35 days old, 150 – 300 g, n = 30) were divided into five groups (n = 6 rats/group): normal control group (K1); control groups induced by DMBA without treatment (K2); treatment group induced by DMBA + sappan wood 0.072 g + *pangdahai* 0.012 g + basil seeds 0.045 g (X1); treatment group induced by DMBA + sappan wood 0.144 g + *pangdahai* 0.024 g + basil seeds 0.09 g (X2); and treatment group induced by DMBA + sappan wood 0.288 g + *pangdahai* 0.048 g + basil seeds 0.18 g (X3). All rats were gently palpated in the mammary glands to detect the development of any abnormal mass after 5 weeks of intervention. A significant increase in TAC levels was observed in the X1, X2, and X3 groups compared with the K2 group after the treatments ( $p = 0.000$ ). In addition, there was a significant decrease in the MDA levels in the X1, X2, and X3 groups compared with the K2 group after the treatments ( $p = 0.000$ ). In conclusion, the administration of *Aia tempayang* increases the TAC levels and decreases the MDA levels in breast cancer rats induced by DMBA.

**1. Introduction**

Breast cancer is the most frequent type of cancer in women all over the world. Breast cancer was diagnosed in more than 2.3 million women worldwide in 2020, with 685,000 fatalities (World Health Organization (WHO) 2021a, World Health Organization (WHO), 2021b). The incidence of breast cancer in women in Indonesia is 42.1 per 100,000 populations with an average death rate of 17 per 100,000 populations (Ministry of Health of The Republic of Indonesia, 2019). Breast cancer occurs due to the abnormal growth and proliferation of cells derived from breast tissue (Khuwaja and Abu-Rezq, 2004). The development of breast cancer is initiated by the presence of a mutated cell that leads to abnormal proliferation.

Furthermore, additional mutations that occur in tumour cells result in tumour progression to accelerated growth and malignancy (Cooper, 2000). Hyperproliferation in breast tissue is caused by continuous stimulation by carcinogenic factors (Sonnenschein and Soto, 2016). Carcinogens are substances that induce cancer by damaging the genome or interfering with cellular metabolic processes. They are classified as chemical agents and physical agents (Pitot, 2002). Chemical carcinogens can affect the expression and activity of certain genes that are responsible for cell growth, differentiation, DNA repair, and apoptosis (Pitot, 2002). It can produce reactive oxygen species (ROS) that cause oxidative stress (Klaunig *et al.*, 2010). Oxidative stress

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contributes to breast cancer where there is an imbalance between oxidants and antioxidants in the body (Sies, 1997). Oxidative stress plays a role in the activation of cell signalling pathways, including apoptosis which is a mechanism in the development and metastasis of cancer (Brown and Bicknell, 2001; Klaunig and Kamendulis, 2004). Increased ROS and oxidative stress are the main characteristics of malignant tumours (Gorrini et al., 2013). Numerous studies have demonstrated that breast cancer can be prevented with antioxidants as they interfere with the metabolic activation of chemical carcinogens (Bagchi and Puri, 1998).

In Indonesia, various kinds of traditional drinks are believed to be efficacious for health, such as *Aia tempayang*. *Aia tempayang* is a traditional drink typical of West Sumatra which comes from sappan wood, *pangdahai*, and basil seeds. The content of each ingredient contains compounds that can prevent breast cancer, such as the content of brazilin in sappan wood which acts as an antioxidant by scavenging free radicals (Bae et al., 2005; Sasaki et al., 2007). Extract of the sappan wood possesses cytotoxic and matrix metalloproteinases (MMPs) inhibitory activities on 4T1 breast cancer cell lines (Haryanti et al., 2018). *Pangdahai* (*Scaphium affine* (Mast.) Pierre) and basil seeds (*Ocimum basilicum*, Linn) contain flavonoids and alkaloids that also exhibit antioxidant and anti-inflammatory activities (Daroi et al., 2015; Jayanti et al., 2016). Flavonoids can act as antioxidants and pro-oxidants in oxidative stress which can activate apoptosis and suppress proliferation and inflammation (Kopustinskiene et al., 2020). Supplementation of 100  $\mu\text{L/kg}$  body weight of basil seed oil has chemopreventive activity against fibrosarcoma tumours (Prakash and Gupta, 2000). Basil seed extracts have cytotoxic activity on human osteosarcoma cell lines (Jayanti et al., 2016). *Pangdahai* has anticancer effects against colorectal cancer cells (Kawk et al., 2021). The role of flavonoids in cancer is by inhibiting the activation of cytochrome p450, where this cytochrome is able to activate procarcinogens to reactivate intermediates that can trigger carcinogenesis that cytochrome p450 tends to have a protective role against the induction of cell damage by carcinogenic activation (Le Marchand et al., 2000; Tsyrllov et al., 1994). Flavonoids also play a role in inhibiting pro-oxidant processes that cause tumour development (Sharma et al., 2011). Brazilin has shown antioxidant activity (Sasaki et al., 2007) which is able to control the redox balance in malignant cells (Nimse and Pal, 2015). An imbalance in cell homeostasis can increase ROS production leading to malignancy and metastasis (Myant et al., 2013; Wang et al., 2015). Alkaloids also exhibit anticancer agents. These compounds can inhibit the development of cancer cells

(Oberlies and Kroll, 2004). Alkaloids show cytotoxic activity by inducing autophagy, necroptosis and apoptosis in the apoptotic pathway (Mondal et al., 2019).

This study aimed to prove the effectiveness of the administration of *Aia tempayang* in preventing breast cancer in rats induced by 7,12-dimethylbenz(a)anthracene (DMBA) by increasing the total antioxidant capacity and decreasing the malondialdehyde level.

## 2. Materials and methods

This study is included in the research project Study of The Administration of *Aia tempayang* (sappan wood, *pangdahai*, and basil seeds) as The Prevention of Breast Cancer in Rats Induced by DMBA.

### 2.1 Samples

This study used brewed *Aia tempayang* as a sample. *Aia tempayang* was obtained from Solok traditional market, West Sumatra, Indonesia. It was stored using aluminium foil to prevent damage to its physical properties during the shipping process from Solok to Semarang. *Aia tempayang* was stored in airtight containers. It made from dried sappan wood, *pangdahai*, and basil seeds. The brewing process of *Aia tempayang* was carried out by mixing all the ingredients according to the dosage variation and then brewing it in water at 70°C for 20 mins as the main substance, brazilin, survives at this temperature

### 2.2 Animal and biological materials

Healthy female Sprague Dawley rats (35 days old, 150 – 300 g, n = 30) were obtained from the Center for Food and Nutrition Studies Gadjah Mada University, Yogyakarta, Indonesia. DMBA was administered via injection of 20 mg.kg<sup>-1</sup> BW.

### 2.3 Laboratory methods

In the examination of TAC levels, the 2,2-difenil-1-pikrilhidrazil (DPPH) method was used, whereas, in the examination of the MDA levels, the thiobarbituric acid reactive substance (TBARS) method was used (BLOIS, 1958; Yagi, 1994). The analysis of antioxidant activity includes the extraction of the active ingredients, namely by pulverizing the sample, weighing it, adding ethanol solvent, centrifuging it, and obtaining the sample supernatant. The next step for antioxidant analysis was to take the sample supernatant and tested it by adding DPPH solution and using a UV spectrophotometer at 517 nm. The working principle of the MDA measurement is the reaction of one molecule of MDA with two molecules of thiobarbituric acid (TBA) to form a pink color which is measured on a spectrophotometer at 532

nm. Blood sampling was performed to analyze the TAC levels and the MDA levels through the plexus retro-orbital. Blood serum was analyzed at the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia.

#### 2.4 Animal treatments

The rats were randomly divided into five groups (n = 6/group): the normal control group (K1) were provided with a standard feed and water ad libitum. The breast cancer control group without treatment (K2) were induced by DMBA and fed with a standard feed and water ad libitum. Treatment group 1 (X1) was administered brewed *Aia tempayang* with a dose simultaneously induced by DMBA, namely, a combination of sappan wood at a dose of 0.072 g.200 g<sup>-1</sup> BW/day, *pangdahai* 0.012 g.200 g<sup>-1</sup> BW/day, basil seeds 0.045 g.200 g<sup>-1</sup> BW/day, and standard feed and water ad libitum. Treatment group 2 (X2) was induced by DMBA and administered brewed *Aia tempayang* with a combination dose of sappan wood at a dose of 0.144 g.200 g<sup>-1</sup> BW/day, *pangdahai* 0.024 g.200 g<sup>-1</sup> BW/day, basil seeds 0.09 g.200 g<sup>-1</sup> BW/day, and standard feed, and water ad libitum. Treatment group 3 (X3) was induced by DMBA and administered brewed *Aia tempayang* with a combination dose of sappan wood at a dose of 0.288 g.200 g<sup>-1</sup> BW/day, *pangdahai* 0.048 g.200 g<sup>-1</sup> BW/day, basil seeds 0.18 g.200 g<sup>-1</sup> BW/day, and standard feed, and water ad libitum. Induction of DMBA was induced through the subcutaneous area of the areola of rats twice a week for 5 weeks of intervention. All rats were gently palpated in the mammary glands to detect the development of any abnormal mass after 5 weeks of intervention.

#### 2.5 Statistical analysis

Statistical analyses were conducted using the SPSS 23 statistics program. Data are expressed as mean±SD. A paired t-test and a one-way analysis of variance were employed for the parametric results; differences between the groups were evaluated using the post hoc test.

### 3. Results and discussion

In this study, we induced DMBA in female rats treated with brewed *Aia tempayang* and evaluated the effectiveness of the administration of *Aia tempayang* in

preventing breast cancer. The results indicated that there was an effect of brewed *Aia tempayang* on the prevention of breast cancer in DMBA-induced breast cancer rats. Gentle palpation of the mammary glands in the treatment groups revealed no abnormal mass development compared to the K2 group which showed multiple nodular masses.

A person suffering from breast cancer may experience weight loss due to several conditions, such as insufficient food consumption, impaired digestion and absorption, and increased energy expenditure (Theologides, 1972). In this study, the K2 group induced by DMBA without treatment-experienced weight loss (-9.00(-10-(-8)) g) during the intervention. Weight loss may occur due to inflammation in rats induced by DMBA. DMBA is a strong carcinogenic agent that has been widely used for research on breast cancer in female Sprague Dawley rats (Huggins et al., 1961). DMBA can cause oxidative damage in various organs, especially the liver and breast (Benakanakere et al., 2010; Vinothini et al., 2009). The DMBA carcinogenicity was accompanied by increasing antioxidant enzyme activity in response to induced oxidative stress and production of reactive oxygen species (Fang et al., 2009). Cancer patients usually experience cachexia, which is loss of weight and loss of muscle mass and function due to increased activity of pro-inflammatory cytokines during cancer development (Argilés et al., 2009; MacDonald et al., 2003; Muscaritoli et al., 2010; Straub et al., 2010). The expression of TNF-α which is a pro-inflammatory cytokine has an anorexic effect and this cytokine induces cachexia (Batista et al., 2012).

The groups that received the intervention of *Aia tempayang* experienced weight gain, namely, the X1 (29.50(28-30) g), X2 (40.00(38-41) g), and X3 (40.50(39-41) g) groups as can be seen from Table 1. The weight gain in the X3 group was not significantly different from that in the K1 group (40.50(39-41) g). Groups X1, X2 and X3 did not experience weight loss even though it was induced with DMBA because these groups were administered *Aia tempayang*. *Aia tempayang* is made from sappan wood, *pangdahai*, and basil seeds, these ingredients contain phytochemicals that function as antioxidants, such as flavonoids, brazilin, and alkaloids which have the potential to maintain body weight (Bertoia et al., 2015).

Table 1. The average body weight before and after treatments.

Body Weight (g)	K1	K2	X1	X2	X3	p'
Pre	175.83±4.58	178.50±3.73	181.00±4.78	182.67±3.55	181.17±5.04	0.090
Post	216.00±4.86	169.67±3.27	210.33±4.55	222.50±3.45	221.33±4.41	0.000
Δ	40.50(39-41)	-9.00(-10-(-8))	29.50(28-30)	40.00(38-41)	40.50(39-41)	0.000 <sup>a</sup>
p	0.000	0.000	0.000	0.000	0.000	

P: Paired t-test, <sup>a</sup>Kruskal Wallis test, p': One Way ANOVA

A significant difference in the TAC levels was observed after the administration of *Aia tempayang* to DMBA-induced rats ( $p = 0.000$ ) as can be seen from Table 2. The TAC levels in the X1 ( $3.28 \pm 0.43 \text{ mmol.mL}^{-1}$ ), X2 ( $4.73 \pm 0.22 \text{ mmol.mL}^{-1}$ ), and X3 ( $5.52 \pm 0.28 \text{ mmol.mL}^{-1}$ ) groups increased compared with the K2 group ( $1.15 \pm 0.11 \text{ mmol.mL}^{-1}$ ) which did not receive brewed *Aia tempayang*. The TAC levels in the X3 group were not significantly different from those in the K1 group ( $5.98 \pm 0.31 \text{ mmol.mL}^{-1}$ ). A significant difference in the MDA levels was observed after the treatments ( $p = 0.000$ ) as can be seen from Table 2. The MDA levels in the K2 group increased ( $9.90 \pm 0.13 \text{ nmol.mL}^{-1}$ ) compared with the K1 group ( $1.78 \pm 0.03 \text{ nmol.mL}^{-1}$ ). The X1 ( $5.02 \pm 0.19 \text{ nmol.mL}^{-1}$ ), X2 ( $2.93 \pm 0.11 \text{ nmol.mL}^{-1}$ ) and X3 ( $1.98 \pm 0.28 \text{ nmol.mL}^{-1}$ ) groups experienced a decrease in the MDA levels because the three groups received brewed *Aia tempayang* treatment. The MDA levels in the X3 group were not significantly different from those in the K1 group. It implies that brewed *Aia tempayang* with a combination dose of sappan wood at a dose of  $0.288 \text{ g.200 g}^{-1} \text{ BW/day}$ , *pangdahai*  $0.048 \text{ g.200 g}^{-1} \text{ BW/day}$ , basil seeds  $0.18 \text{ g.200 g}^{-1} \text{ BW/day}$  is the most effective dose to prevent breast cancer compared with other doses.

Malondialdehyde (MDA) is the end product of oxidative injury, which is an indicator of the level of cell damage caused by free radicals (Mihás et al., 2003; Yang, et al., 2008). DMBA induction in rat models of breast cancer increased the MDA and total nitrate levels (Batcioglu et al., 2012). The increase in the MDA levels is caused by an increase in lipid peroxide in the cell membrane (Moselhy and Al mslmani, 2008; Selamoglu Talas et al., 2009). Previous studies have also demonstrated that increased MDA levels occur in breast tumour tissue in mammals (Kumaraguruparan et al., 2005). Elevated MDA is one of the biomarkers of oxidative stress. When MDA increases, the production of reactive oxygen species (ROS) becomes excessive, or the antioxidants in the body are reduced; even these conditions can simultaneously occur in the body (McMichael, 2007).

*Aia tempayang* is made from sappan wood, *pangdahai*, and basil seeds. Sappan wood contains various phytochemical compounds, such as flavonoids, phenolics, brazilin, tannins, and saponins (Niu et al.,

2020; Saravanakumar and Chandra, 2013). *Pangdahai* contains lignans, phenylpropanoids, flavonoids, alkaloids, phenolics, glycosides, peptides, and phenolic acids (Oppong et al., 2020; Yang et al., 2016). Flavonoids, alkaloids, tannins, and terpenoids are contained in basil seeds (Jayanti et al., 2016). The content of phytochemicals in *Aia tempayang* act as antioxidants.

The results of collaboration research showed that flavonoids found in *Aia tempayang* (Widyastuti and Anjani, 2021) may decrease the serum levels of MDA and increase the TAC levels (Alipour et al., 2016; Rasyid et al., 2012). This compound can also prevent the inflammatory response caused by oxidative stress by inhibiting interleukin (IL)-1 $\beta$ , IL-6, and tumour necrosis factor (TNF)- $\alpha$  (Zeng et al., 2020). Brazilin is a water-soluble flavonoid contained in sappan wood. It exhibits antioxidant activity through its ability to scavenge free radicals as well as ferric reduction activity. The structure of dibenzoxocin in this compound is effective in scavenging free radicals (Sasaki et al., 2007). In addition, brazilin exhibits an anti-inflammatory activity that inhibits the production of NO, a toxic gas associated with various diseases, such as atherosclerosis, inflammation, and carcinogenesis (Jeong et al., 2008; Sasaki et al., 2007). Brazilin in sappan wood reduces the MDA levels in rats by scavenging OH radicals (Hu et al., 2008; Safitri et al., 2016). Sappan wood extract significantly inhibits MDA formation and hydrogen peroxide radical scavenging (Hu et al., 2008).

Previous research explained that *pangdahai* exhibits an antioxidant activity that can scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals and reduce potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (Daroi et al., 2015). Alkaloid compounds contained in *pangdahai* are also capable of reducing MDA levels (Liu et al., 2019). *Pangdahai* exhibits an anticancer activity, and previous studies have demonstrated that *S. affine* ethanol extracts (SAE) were able to reduce tumour size in the SAE group compared with the control group. Tumour size also decreases with the increase in SAE concentrations (Kawk et al., 2021). The alkaloid extract can increase the total antioxidant capacity in rabbits. Alkaloids can also reduce lipid peroxidation in tissues, indicating their antioxidant activity that can reduce oxidative stress. TAC describes the total antioxidant characteristics of all antioxidants

Table 2. The TAC levels dan the MDA levels after treatments

Groups	K1	K2	X1	X2	X3	<i>p</i>
TAC levels ( $\text{mmol.mL}^{-1}$ )	$5.98 \pm 0.31^a$	$1.15 \pm 0.11^b$	$3.28 \pm 0.43^c$	$4.73 \pm 0.22^d$	$5.52 \pm 0.28^a$	0.000*
MDA levels ( $\text{nmol.mL}^{-1}$ )	$1.78 \pm 0.03^a$	$9.90 \pm 0.13^b$	$5.02 \pm 0.19^c$	$2.93 \pm 0.11^d$	$1.98 \pm 0.28^a$	0.000*

Values are presented as mean $\pm$ SD. Values with different notations within the same row are statistically significantly different. *p*: One Way ANOVA post hoc Tukey test.

found in plasma (Al-Fartosy *et al.*, 2013).

Basil seed extract exhibits a strong antioxidant activity that reduces DPPH, NO, and OH radicals and neutralizes H<sub>2</sub>O<sub>2</sub> (Kaurinovic *et al.*, 2011). The administration of basil seeds along with the induction of deltamethrin in rats led to a decrease in the MDA levels compared with the control group that did not receive basil seed intervention (Sakr and Al-Amoudi, 2012). Previous research on sensitized mice demonstrated that the administration of basil seed extract can reduce the levels of MDA, NO<sub>2</sub>, NO<sub>3</sub> and WBC and increase the serum levels of antioxidants (Eftekhar *et al.*, 2018). Basil seeds are capable of preventing the development of fibrosarcoma tumors and reducing tumour volume at a dose of 100 µL.kg<sup>-1</sup> of body weight (Prakash and Gupta, 2000).

#### 4. Conclusion

The administration of *Aia tempayang* to DMBA-induced rats can prevent breast cancer in rats by reducing the MDA levels and increasing the TAC levels. The treatment groups revealed no abnormal mass development in the mammary glands after the intervention. *Aia tempayang* with a combination dose of sappan wood at a dose of 0.288 g.200 g<sup>-1</sup> BW/day, *pangdahai* 0.048 g.200 g<sup>-1</sup> BW/day, basil seeds 0.18 g.200g<sup>-1</sup> BW/day is the most effective dose to prevent breast cancer.

#### Conflict of interest

The authors declare no conflict of interest.

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