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## Preface

The International Conference 2021 (INAFOR 2021 Stream 2) was held on 7-8 September 2021 in a virtual format via zoom meetings due to the COVID-19 pandemic situation. It was conducted virtually to align with the biannual agenda of The 6th International Conference of Indonesia Forestry and Environment Researchers (INAFOR), which previously was carried out by The Agency for Standardization of Environment and Forestry Instruments, Ministry of Environment of Forestry, Indonesia. This is the first international conference hosted by The Center for Standardization of Sustainable Forest Management Instruments, Bogor, West Java, Indonesia. This conference was an important medium for sharing information and experiences and encouraging collaboration in sustainable forest management.

The theme "Managing Forest and Natural Resources, Meeting Sustainable and Friendly Use", strategically supports Indonesia's commitment to Net Sink Forestry and Land Use (FoLU) 2030. The INAFOR 2021 Stream 2 was attended by approximately 750 participants, invitees, keynote speakers, scientists, and academicians from Indonesia, South Korea, the Netherland and Australia. The conference took place with a plenary session featuring six Keynote Speakers that presented exciting and practical information relevant to the theme. Also, a parallel session was divided into 12 separate zoom spaces according to the topics and number of participants. The presenters had ten minutes to present their papers and followed by interactive and engaging discussion.

This proceeding presents 78 papers of research results on various topics, including biodiversity conservation, livelihoods, climate resilience, timber, and non-timber forest products. Those valuable pieces of information and recommendations can be modalities and references for the preparation and development of standards for sustainable forest management instruments and the development of science, technology, and innovation.

Thank you.

Dr. Wening Sri Wulandari  
Acting Director of Center for Standardization of Sustainable Forest Management  
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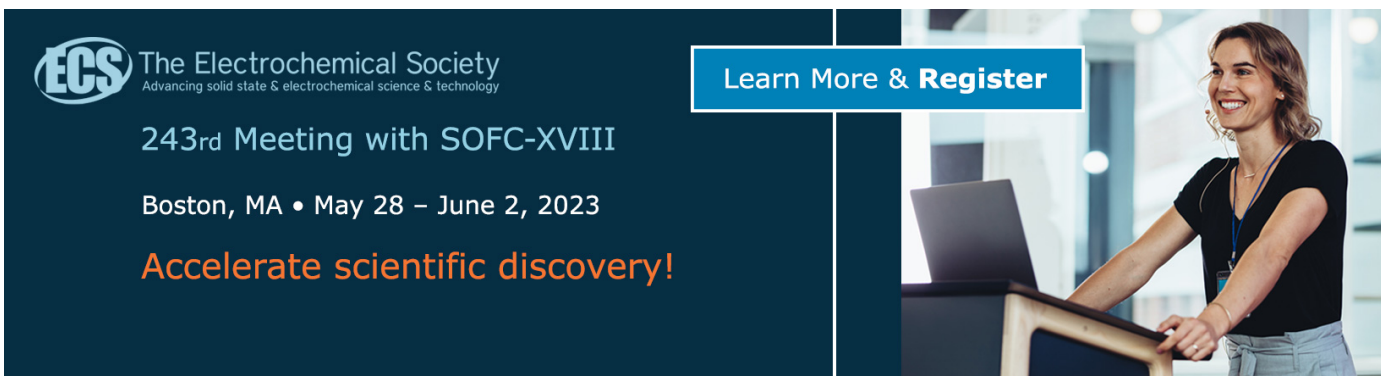
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# Total phenolic, antioxidant activity and toxicity of taxus leaf herbal tea in two different brewing temperature

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**Abstract.** Taxus has been recognized to have many medicinal properties. This study aims to determine the total phenolic, antioxidant activity, toxicity effect of taxus leaf herbal tea from two brewing temperatures. Antioxidant test was carried out by IC<sub>50</sub>-DPPH, total phenolic was carried out by spectrophotometric analysis, and toxicity effect was measured with BSLT (Brine Shrimp Lethality Test) method. The taxus leaves were prepared by drying method at 60°C until the water content reached 10% and then ground until they were quite smooth. Brewing was carried out at a temperature of 75°C and 95°C. The results showed that taxus leaves had strong antioxidants with IC<sub>50</sub> values reaching 62.5 ppm and total phenols of 15.68% (w/w) and 14.08% (w/w) at each brewing temperature. The LC<sub>50</sub> of toxicity effect of taxus leaves at 290.58 ppm and 536.38 ppm. We concluded the taxus leaves was a good natural antioxidant source with phenol content and could be develop to natural drug for anticancer.

## 1. Introduction

Herbal teas have been recognized for a long time and the demand is getting higher along with the awareness to return to nature. Herbal teas or tisane is herbal infusion which is made from other than *Camellia sinensis* leaves [1] and could be made from various parts of plants, including leaves, flowers, fruit, bark and roots [2]. The benefits of this herbal tea vary, but one of the most common benefits is the presence of antioxidants [3]. These antioxidants function in protecting the body from exposure to free radicals so that they can reduce the risk of developing cancer [4].

*Taxus sumatrana* Miq. de Laub. is one of plant species that has many benefits. Taxus is member of the Taxaceae family which is capable of producing paclitaxel which functions as a cancer cell growth inhibitor in humans [5]. At present, paclitaxel is one of the most important anti-cancer drugs for treating ovarian cancer and breast cancer as well as being promising in treating neck cancer, lung cancer, gastrointestinal cancer and bladder cancer [6], glandular cancer lymph and several other types of cancer [7]. The use of this type as a cancer drug has been quite extensive, especially from the types of *Taxus baccata* L. [8].



Taxus species found in Indonesia is the type of *Taxus sumatrana* Miq. de Laub. and spread in North Sumatra, South Sumatra, Lampung and Sulawesi [9]. *Taxus sumatrana* is potential for cancer drugs, and it has been studied to treat various types of cancers such as human liver carcinoma (Hepa59T/VGH), human large cell carcinoma of the lungs (NCI), human cervical epithelioid carcinoma (HeLa), human colon adenocarcinoma (DLD-1), human medulloblastoma (Med) cell lines, human PC-3 tumor cells [10-12].

Cancer is one of the most common non-infectious diseases in the world. Based on WHO data, in 2020 there have been deaths of nearly 10 million people worldwide. The highest mortality is caused by breast cancer [13]. Meanwhile, there are around 1 million cancer sufferers in Indonesia and each year this number is increasing [14]. Cancer is a cell that grows abnormally. Cancer cell growth can run continuously without control. Apoptosis regulation, namely the process of controlled cell death from cancer cells not functioning normally as normal cells. In the metastatic stage, cancer cells can spread from one tissue to another or from one organ to another. When this happens, the patient's condition will worsen because there are many types of cancer in the patient's body [13].

Cancer can be caused by various factors such as infection from viruses and chemical compounds that can damage the body's biochemical compounds such as free radicals. The damage caused by free radicals can cause damage to the cell's life cycle system, because it can cause mutations in DNA [15]. Free radicals can be generated from metabolic processes and can come from outside the body. Therefore, we need compounds that can neutralize free radicals, namely antioxidants. There are 2 groups of sources of antioxidants, namely endogenous and exogenous. Endogenous antioxidants are enzymes that can catalyze free radical reactions into relatively stable compounds such as catalase and glutathione peroxidase. Meanwhile, exogenous antioxidants can be in the form of natural ingredients such as flavonoids, alkaloids and terpenoids [17,18].

This research was conducted to understand the potential of herbal tea from taxus leaves as a source of antioxidants and raw material for anticancer drugs, it is necessary to carry out several bioactivity tests. These tests were in the form of antioxidant test using the DPPH method (2,2-diphenyl-1-picrylhydrazyl), total phenolic content test, and toxicity test using the BSLT method. The purpose of this study was to assess the antioxidants, phenolic compounds, and the toxicity value of taxus herbal tea.

## 2. Materials and Methods

### 2.1. *Taxus* herbal tea preparation

The harvested taxus leaves were dried first under the sun for 24 hours then put in the oven at 60°C. Dry leaves were characterized by a moisture content of less than 10%. These leaves were then crushed using a blender to the size of about 1-3 mm then put into a tea bag weighing 1.5 g per bag.

### 2.2. *Samples test preparation*

The teabags were brewed in two temperature variations, which were 75°C and 95°C. About 100 mL of brewed water was used and then evaporated until the extract remains. The extract was then tested according to the specified test parameters.

### 2.3. *Antioxidant analysis*

First, DPPH 125 µM stock preparation. DPPH weighed as much as 2.5 mg. Dissolved with ethanol p.a into a volumetric flask. Squeezed up to a volume of 50 mL. Aluminum foil coated flask. DPPH is ready to use. Second, sample preparation and vitamin C. Weighing sample and vitamin C, each weighing as much as 10 mg. Then dissolved in 1 mL of DMSO. Sonicated until dissolved, then vortexed. Sample and vitamin C were ready to use.

Procedure: enter a sample of 100 µL into the microplate. 100 µL of DPPH were added for sample replications 1 and 2, while for negative control only 100 µL of p.a ethanol were added. After that, it was



incubated at room temperature in the dark for 30 minutes. Then measured in a spectrophotometer at a wavelength of 517 nm.

#### 2.4. Total phenolic analysis

Standard (gallic acid) stock preparation. Gallic acid main solution was made with a concentration of 500 ppm, weighed as much as 12.5 mg and dissolved with methanol p.a in a 25 mL volumetric flask. Then make a standard concentration range of 0; 10; 30; 50; 70; and 100 ppm in a 25 mL flask. Pipette 1 mL of gallic acid solution each into the test tube. Then 5 mL of 7.5% Folin Ciocalteu reagent was added, vortexed and incubated in the dark room for  $\pm 8$  minutes. After that, 4 mL of 1% NaOH was added, vortexed and incubated in a dark room for 1 hour. Measured using a spectrophotometer at a wavelength of 730 nm. Sample: weighed 10 mg of extract or pipette as much as 5 mL of liquid into a 25 mL volumetric flask and dissolved with methanol p.a.

Pipette as much as 1 mL into the test tube. Then 5 mL of 7.5% Folin Ciocalteu reagent was added, vortexed and incubated in the dark room for  $\pm 8$  minutes. After that, 4 mL of 1% NaOH was added, vortexed and incubated in a dark room for 1 hour. Measured using a spectrophotometer at a wavelength of 730 nm.

#### 2.5 Toxicity analysis (BSLT method)

This method uses *Artemia salina* L. as the object of testing the toxic effects of herbal taxus tea. A salina eggs were hatched in a container that has an aerator and have been given a bulkhead in the middle of the container and contain 500 mL of salt water. One part was closed to make it darker while the other side is left without cover. The BSLT test was carried out in a bright place. *Artemia salina* L. eggs within 48 hours will hatch into larvae (nauplii).

*Artemia salina* L. larvae that had hatched within 48 hours were transferred to a test tube. Each test tube contains 10 larvae. Then, each tube containing the larvae was added with taxus herbal tea and incubated for 24 hours. After that, the percentage of larval mortality caused by taxus herbal tea was calculated and the LC<sub>50</sub> value was calculated.

### 3. Results and Discussion

#### 3.1. Antioxidant

The test results showed that both samples had antioxidant activity (Table 1). The IC<sub>50</sub> value obtained was the same, which is 62.50 ppm. This value indicates that herbal tea brewed at a temperature of 75°C and 95°C is able reduce DPPH as free radicals as much as 50% at a concentration of 62.50 ppm. DPPH compounds are free radicals that are stable at room temperature and are purple in color. If there are other compounds that can donate electrons to the DPPH compound, the solution will change color from purple to yellow. DPPH level measurements were carried out at a wavelength of 517 nm because at that wavelength it has the maximum absorption for the purple DPPH color. The change in color from purple to yellow is directly proportional to the number of electrons captured or shared between DPPH and antioxidant compounds [18]. When compared with other teas such as green tea, white tea, and oolong tea from the Chinese region, the antioxidant activity of taxus herbal tea is lower by half [19]. However, this activity is considered good because low concentrations can reduce free radicals by as much as 50%.

**Table 1.** Antioxidant, total phenolic and LC<sub>50</sub> of taxus herbal tea.

Water Temperature (°C)	Antioxidant IC <sub>50</sub> -DPPH (ppm)	Total Phenolic (% w/w)	LC <sub>50</sub> (ppm)
75	62.50	15.68	290.58
95	62.50	14.08	536.38

### 3.2. Total phenolic

The results of the total phenol test showed differences in the phenol content contained in each sample (Table 1). Herbal teas brewed at 75°C showed higher phenol content (15.68%) than those brewed at 95°C (14.08%). Although the phenol levels in the two samples were different, their antioxidant activity was not different. Phenolic compounds such as flavonoids are known to be one of the phytochemicals that have antioxidant activity [17]. The mechanism that can occur from phenolic compounds as antioxidants is by giving or sharing electrons from the hydroxyl group of the phenol compound with free radical compounds [20]. Some phenol compounds will be damaged at high temperatures, this can cause differences in phenol levels in the two samples [21]. Therefore, when testing phenol levels using Folin Ciocalteu reagent, the reagent can no longer oxidize the hydroxyl group of the phenol compound so that it is not detected by the reagent.

The reaction principle of determining phenol content with Folin Ciocalteu reagent is to oxidize the hydroxyl group of the phenol compound being tested [22]. The assumption obtained from the antioxidant activity test and total phenol content test in these two herbal teas is that the antioxidant activity obtained in these two samples does not only depend on the phenolic compounds contained, there are other compounds in these herbal teas that have antioxidant activity. Phenol levels in taxus herbal tea can be said to be low when compared to phenol levels in other tea drinks such as Zesta green tea, Lipton green tea, and Lipton black tea (60-75%). However, when compared to its phenol content with fresh *Cymbopogon citarus* (10%), the phenol content of taxus herbal tea is 5% higher [23].

### 3.3. Toxicity

The results of the toxicity test showed differences in the toxic effects of the two samples (Table 1). Taxus herbal teas brewed at 75°C showed greater toxic effects (290.58 ppm) than those brewed at 95°C (536.38 ppm). The LC<sub>50</sub> value obtained in taxus herbal tea brewed at 75°C indicates that at a concentration of 290.58 ppm, the tea can kill as much as 50% of the population of *Artemia salina* larvae used in the BSLT test. Whereas in taxus herbal tea brewed at 95°C, the mortality of 50% of the larva population occurred at a concentration of 536.38 ppm.

The BSLT method has been widely used to show the activity of compounds contained in natural materials. This test has the advantage of fast analysis execution time as a bioactivity test at an early stage [24]. Based on the LC<sub>50</sub> value obtained, taxus herbal tea brewed at a temperature of 75°C and 95°C can be categorized as having a good content of active compounds. A natural material is categorized as having a good content of active compounds if when tested for bioactivity it has an LC<sub>50</sub> value below 1000 ppm, if the LC<sub>50</sub> value is above 1000 ppm then the natural material is categorized as having no active compound. The toxic effect shown by the results of this analysis does not indicate that this herbal tea is not suitable for consumption. The toxic value of this analysis only shows that the compounds contained in these herbal teas are compounds that have bioactivity and become an initial stage of research with the aim of developing anticancer phytopharmaca [25]. This can happen because the main object of the BSLT method is shrimp larvae, which are organisms that can be categorized as simple organisms. In addition, the unit of toxicity used in this method is ppm (parts per million), which is a unit of concentration that shows a small amount so that further testing and the use of a larger concentration is needed if you want to see the toxic effect on humans.

#### 4. Conclusion

This taxus herbal tea has quite strong antioxidant activity. This activity is obtained because the herbal tea contains phenolic compounds. This herbal tea can also be potential as a supportive therapy for anticancer treatment. However, it is necessary to do further studies in vitro and in vivo.

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*by A D Sunandar, M Alfarabi*

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Cancer is one of the most common non-infectious diseases in the world. Based on WHO data, in 2020 there have been deaths of nearly 10 million people worldwide. The highest mortality is caused by breast cancer [13]. Meanwhile, there are around 1 million cancer sufferers in Indonesia and each year this number is increasing [14]. Cancer is a cell that grows abnormally. Cancer cell growth can run continuously without control. Apoptosis regulation, namely the process of controlled cell death from cancer cells not functioning normally as normal cells. In the metastatic stage, cancer cells can spread from one tissue to another or from one organ to another. When this happens, the patient's condition will worsen because there are many types of cancer in the patient's body [13].

Cancer can be caused by various factors such as infection from viruses and chemical compounds that can damage the body's biochemical compounds such as free radicals. The damage caused by free radicals can cause damage to the cell's life cycle system, because it can cause mutations in DNA [15]. Free radicals can be generated from metabolic processes and can come from outside the body. Therefore, we need compounds that can neutralize free radicals, namely antioxidants. There are 2 groups of sources of antioxidants, namely endogenous and exogenous. Endogenous antioxidants are enzymes that can catalyze free radical reactions into relatively stable compounds such as catalase and glutathione peroxidase. Meanwhile, exogenous antioxidants can be in the form of natural ingredients such as flavonoids, alkaloids and terpenoids [17,18].

This research was conducted to understand the potential of herbal tea from taxus leaves as a source of antioxidants and raw material for anticancer drugs, it is necessary to carry out several bioactivity tests. These tests were in the form of antioxidant test using the DPPH method (2,2-diphenil-1-picrylhydrazyl), total phenolic content test, and toxicity test using the BSLT method. The purpose of this study was to assess the antioxidants, phenolic compounds, and the toxicity value of taxus herbal tea.

## 2. Materials and Methods

### 2.1. *Taxus* herbal tea preparation

The harvested taxus leaves were dried first under the sun for 24 hours then put in the oven at 60°C. Dry leaves were characterized by a moisture content of less than 10%. These leaves were then crushed using a blender to the size of about 1-3 mm then put into a tea bag weighing 1.5 g per bag.

### 2.2. Samples test preparation

The teabags were brewed in two temperature variations, which were 75°C and 95°C. About 100 mL of brewed water was used and then evaporated until the extract remains. The extract was then tested according to the specified test parameters.

### 2.3. Antioxidant analysis

First, DPPH 125 µM stock preparation. DPPH weighed as much as 2.5 mg. Dissolved with ethanol p.a into a volumetric flask. Squeezed up to a volume of 50 mL. Aluminum foil coated flask. DPPH is ready to use. Second, sample preparation and vitamin C. Weighing sample and vitamin C, each weighing as much as 10 mg. Then dissolved in 1 mL of DMSO. Sonicated until dissolved, then vortexed. Sample and vitamin C were ready to use.

Procedure: enter a sample of 100 µL into the microplate. 100 µL of DPPH were added for sample replications 1 and 2, while for negative control only 100 µL of p.a ethanol were added. After that, it was



incubated at room temperature in the dark for 30 minutes. Then measured in a spectrophotometer at a wavelength of 517 nm.

#### 2.4. Total phenolic analysis

Standard (gallic acid) stock preparation. Gallic acid main solution was made with a concentration of 500 ppm, weighed as much as 12.5 mg and dissolved with methanol p.a in a 25 mL volumetric flask. Then make a standard concentration range of 0; 10; 30; 50; 70; and 100 ppm in a 25 mL flask. Pipette 1 mL of gallic acid solution each into the test tube. Then 5 mL of 7.5% Folin Ciocalteu reagent was added, vortexed and incubated in the dark room for  $\pm 8$  minutes. After that, 4 mL of 1% NaOH was added, vortexed and incubated in a dark room for 1 hour. Measured using a spectrophotometer at a wavelength of 730 nm. Sample: weighed 10 mg of extract or pipette as much as 5 mL of liquid into a 25 mL volumetric flask and dissolved with methanol p.a.

Pipette as much as 1 mL into the test tube. Then 5 mL of 7.5% Folin Ciocalteu reagent was added, vortexed and incubated in the dark room for  $\pm 8$  minutes. After that, 4 mL of 1% NaOH was added, vortexed and incubated in a dark room for 1 hour. Measured using a spectrophotometer at a wavelength of 730 nm.

#### 2.5 Toxicity analysis (BSLT method)

This method uses *Artemia salina* L. as the object of testing the toxic effects of herbal taxus tea. A salina eggs were hatched in a container that has an aerator and have been given a bulkhead in the middle of the container and contain 500 mL of salt water. One part was closed to make it darker while the other side is left without cover. The BSLT test was carried out in a bright place. *Artemia salina* L. eggs within 48 hours will hatch into larvae (nauplii).

*Artemia salina* L. larvae that had hatched within 48 hours were transferred to a test tube. Each test tube contains 10 larvae. Then, each tube containing the larvae was added with taxus herbal tea and incubated for 24 hours. After that, the percentage of larval mortality caused by taxus herbal tea was calculated and the LC<sub>50</sub> value was calculated.

### 3. Results and Discussion

#### 3.1. Antioxidant

The test results showed that both samples had antioxidant activity (Table 1). The IC<sub>50</sub> value obtained was the same, which is 62.50 ppm. This value indicates that herbal tea brewed at a temperature of 75°C and 95°C is able reduce DPPH as free radicals as much as 50% at a concentration of 62.50 ppm. DPPH compounds are free radicals that are stable at room temperature and are purple in color. If there are other compounds that can donate electrons to the DPPH compound, the solution will change color from purple to yellow. DPPH level measurements were carried out at a wavelength of 517 nm because at that wavelength it has the maximum absorption for the purple DPPH color. The change in color from purple to yellow is directly proportional to the number of electrons captured or shared between DPPH and antioxidant compounds [18]. When compared with other teas such as green tea, white tea, and oolong tea from the Chinese region, the antioxidant activity of taxus herbal tea is lower by half [19]. However, this activity is considered good because low concentrations can reduce free radicals by as much as 50%.

**Table 1.** Antioxidant, total phenolic and LC<sub>50</sub> of taxus herbal tea.

Water Temperature (°C)	Antioxidant IC <sub>50</sub> -DPPH (ppm)	Total Phenolic (% w/w)	LC <sub>50</sub> (ppm)
75	62.50	15.68	290.58
95	62.50	14.08	536.38

### 3.2. Total phenolic

The results of the total phenol test showed differences in the phenol content contained in each sample (Table 1). Herbal teas brewed at 75°C showed higher phenol content (15.68%) than those brewed at 95°C (14.08%). Although the phenol levels in the two samples were different, their antioxidant activity was not different. Phenolic compounds such as flavonoids are known to be one of the phytochemicals that have antioxidant activity [17]. The mechanism that can occur from phenolic compounds as antioxidants is by giving or sharing electrons from the hydroxyl group of the phenol compound with free radical compounds [20]. Some phenol compounds will be damaged at high temperatures, this can cause differences in phenol levels in the two samples [21]. Therefore, when testing phenol levels using Folin Ciocalteu reagent, the reagent can no longer oxidize the hydroxyl group of the phenol compound so that it is not detected by the reagent.

The reaction principle of determining phenol content with Folin Ciocalteu reagent is to oxidize the hydroxyl group of the phenol compound being tested [22]. The assumption obtained from the antioxidant activity test and total phenol content test in these two herbal teas is that the antioxidant activity obtained in these two samples does not only depend on the phenolic compounds contained, there are other compounds in these herbal teas that have antioxidant activity. Phenol levels in taxus herbal tea can be said to be low when compared to phenol levels in other tea drinks such as Zesta green tea, Lipton green tea, and Lipton black tea (60-75%). However, when compared to its phenol content with fresh *Cymbopogon citratus* (10%), the phenol content of taxus herbal tea is 5% higher [23].

### 3.3. Toxicity

The results of the toxicity test showed differences in the toxic effects of the two samples (Table 1). Taxus herbal teas brewed at 75°C showed greater toxic effects (290.58 ppm) than those brewed at 95°C (536.38 ppm). The LC<sub>50</sub> value obtained in taxus herbal tea brewed at 75°C indicates that at a concentration of 290.58 ppm, the tea can kill as much as 50% of the population of *Artemia salina* larvae used in the BSLT test. Whereas in taxus herbal tea brewed at 95°C, the mortality of 50% of the larva population occurred at a concentration of 536.38 ppm.

The BSLT method has been widely used to show the activity of compounds contained in natural materials. This test has the advantage of fast analysis execution time as a bioactivity test at an early stage [24]. Based on the LC<sub>50</sub> value obtained, taxus herbal tea brewed at a temperature of 75°C and 95°C can be categorized as having a good content of active compounds. A natural material is categorized as having a good content of active compounds if when tested for bioactivity it has an LC<sub>50</sub> value below 1000 ppm, if the LC<sub>50</sub> value is above 1000 ppm then the natural material is categorized as having no active compound. The toxic effect shown by the results of this analysis does not indicate that this herbal tea is not suitable for consumption. The toxic value of this analysis only shows that the compounds contained in these herbal teas are compounds that have bioactivity and become an initial stage of research with the aim of developing anticancer phytopharmaca [25]. This can happen because the main object of the BSLT method is shrimp larvae, which are organisms that can be categorized as simple organisms. In addition, the unit of toxicity used in this method is ppm (parts per million), which is a unit of concentration that shows a small amount so that further testing and the use of a larger concentration is needed if you want to see the toxic effect on humans.

#### 4. Conclusion

This taxus herbal tea has quite strong antioxidant activity. This activity is obtained because the herbal tea contains phenolic compounds. This herbal tea can also be potential as a supportive therapy for anticancer treatment. However, it is necessary to do further studies in vitro and in vivo.

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