

# Sappan Wood Extract Used as Preservative in Chili Paste

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## Abstract

The study was conducted to evaluate the antioxidant activity and the antimicrobial property of sappan heartwood (*Caesalpinia sappan* L.) water extract as natural food preservative. The antimicrobial activities (MIC, MBC and MFC) were evaluated using the 96-well microdilution broth method. Antioxidant activities were determined using the free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. The MICs of sappan wood extract 1 obtained from the antimicrobial activity testing against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Candida albicans* were 250, 125, 500 and more than 16,000 µg/ml, respectively, and were higher than MICs of those sappan wood extracts 2, 3 and 4. The solubility of sappan wood extract 1 was also well soluble. The sappan wood extracts at 2, 4, 8 times of the selected maximum MIC (500 µg/ml) were added to chili paste a favorite Thai food, and were packed in two kinds of utensil (glass and plastic) with covers in order to determine the preservative property up to 12 months (and 3 months repeated). From the total aerobic and fungal count in chili paste, it could be concluded that sappan wood extract could inhibit bacteria but not fungal. Therefore, sappan wood extract could be used as a preservative in food to inhibit bacterial growth for 6 months. Sappan wood extract at the concentration of 2 times of the MIC value can be used as a food preservative. The total aerobic count of chili paste packed in plastic utensils and glass utensils was variable. Therefore, it was difficult to conclude whether plastic or glass utensils would be appropriate to be used for chili paste products. Sappan wood extracts 1, 2, 3 and 4 provided 50 % inhibition (IC<sub>50</sub>) at the concentration of 23.19, 38.30, 29.82 and 71.54 µg/ml, respectively, while ascorbic acid and trolox showed IC<sub>50</sub> at the concentration of 17.11 µg/ml and 24.27 µg/ml, respectively. Sappan wood extract also show promise to be a good antioxidant, which will increase the value of products and be useful for health.

**Key words:** *Caesalpinia sappan* L.; chili paste; preservative; antimicrobial activities; antioxidant activity

## INTRODUCTION

Chili paste is the popular food of Thai people. There are many types of chili paste sold in the market but the problem is that they can easily be spoiled by microorganisms. Because the components of chili paste abound with the nutrients that are appropriate for the growth of the microorganisms for example bacteria, fungal and yeast. The present microorganisms will be originated from the natural microflora

of the raw materials, processing and storage.<sup>1</sup> Contaminated microorganisms come from the raw materials are various mold and yeasts and specific bacteria genera *Bacillus*, *Clostridium*, *Enterobacter*, *Escherichia*, *Micrococcus*, *Proteus*, *Streptococcus* and *Acetobacter*.<sup>2</sup>

Preservation is based firstly on the delay or prevention of microbial growth.<sup>3</sup> Using the chemical preservative agents is concerned about the safety. Hence, there has been recent interest in testing natural

products, including plant-derived compounds, for antimicrobial properties as these may be used as natural preservatives in foods.<sup>4</sup> *Caesalpinia sappan*, which has antimicrobial activity, has long been used in the oriental folk medicines.<sup>5</sup> Therefore, in this report, sappan wood extracts were studied as preservatives by focusing on their antimicrobial activities and food preservative abilities in chili paste.

Moreover, there has been increasing interest in recent years in healthy life styles and healthy aging; correspondingly, interest in antioxidant and food supplement has grown remarkably. Damage mediated by free radicals cause the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA and alteration of platelet functions. It has generally been considered to be linked with many chronic health problems such as cancers, inflammation, aging and atherosclerosis. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in the prevention of degenerative diseases.<sup>6</sup> The interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to various diseases. The search for natural antioxidants and other preparations of plant origin to achieve this objective has been intensified. Many plants are reported to possess antioxidative free radical scavenging.<sup>7</sup> Hence, in this study, free radical scavenging activity of sappan wood extracts was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assay.

## MATERIALS AND METHODS

### *Preparation of Sappan wood extracts*

Sappan wood extracts dried by freeze dry (sample 1 and 2), Sappan wood extract dried by drum dry (sample 3) and Sappan wood extract dried by water bath (sample 4). All of sappan wood extracts were supported by Assoc. Prof. Rungravi Temsiririrkkul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.

### *Determination of Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC) and Minimum Fungicidal Concentrations (MFC) of sappan wood extracts*

The antimicrobial activity testing of all sappan wood extracts against *E. coli* ATCC 25922, *S. aureus* ATCC 6538, *S. typhimurium* ATCC 13311 and *C. albicans* ATCC 10231 were employed by microdilution broth method which was modified from the proposed NCCLS method (M7-A5)<sup>8</sup> in order to determine MIC, MBC and MFC.

Sappan wood extracts were dissolved in water. The 96 well microtiter plates containing a series of two fold dilution of each sample were prepared using Mueller-Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for *C. albicans* as diluent. Inoculums of approximately  $5 \times 10^5$  colony forming unit (CFU/ml) for bacteria and  $5 \times 10^3$  CFU/ml for *C. albicans* were used. Microorganisms were prepared from overnight-stand culture in MHB for bacteria and in SDB for *C. albicans*. Plates were incubated at 37 °C for 16-20 hours for bacteria and 48 hours for *C. albicans* before MIC was observed by the unaided eye. The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of organism in the microdilution wells. Chloramphenicol (Biolab, Thailand) and ketoconazole (T.O. chemicals, Thailand) were used as positive controls.

To determine MBC and MFC, 10 µl of broth was taken from each well and spotted the inoculate on Mueller-Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for *C. albicans*. The numbers of microorganisms were determined after aerobic incubation overnight at 37°C. MBC and MFC are the concentration which is less than 0.1% of the initial inoculum survived. All tests were performed in triplicate.

Sappan wood extract which gave the lowest MIC (best antimicrobial activity) and good solubility in water was selected for the study of food preservative ability in chili paste.

### ***Food preservative ability study of the sappan wood extract in chili paste***

Sappan wood extract (from part 1) was added to the chili paste produced by the Cooperative, Pathumthani province, by adding sappan wood extract at 2, 4 and 8 times of the MIC value (using water as the solvent to dissolve sappan wood extract). Chili paste added with the same amount of water was used as control. Chili paste was packed in two kinds of utensils with covers, glass and plastic, to compare the microbial preventive and preservative activities in these chili paste products. The tests for microbiological quality, moisture content and physiological appearance of chili pastes were determined every month for one year. All samples were stored at room temperature.

### ***Test for microbiological quality***

In this study, the chili paste products were tested for microbiological quality according to the standard recommended by the Department of Medical Science, Ministry of Public Health as following.

Total Aerobic Count < 10<sup>6</sup> CFU/g, MPN Coliforms < 500 MPN/g, MPN *E.coli* < 3 MPN/g, *Staphylococcus aureus* < 100 CFU/g, *Bacillus cereus* < 100 CFU/g, *Salmonella* not found /25 g, *Clostridium perfringens* not found / 0.01 g.

The method used to test for microbiological quality was modified from proposed Bacteriological Analytical Manual (BAM).<sup>9</sup>

### ***Moisture content***

The moisture content of chili paste was determined by drying 5 g of chili paste at 100 °C for 4 hours, placed in the desiccator and weighed subsequently. It was repeatedly done until its weight became constant. The moisture was calculated as the percent loss in weight after drying.<sup>10</sup>

### ***Physiological appearance of the chili paste***

The character, smell and color of chili paste were observed and photographs were taken.

Study of food preservative ability of the sappan wood extract in chili paste was repeated by adding sappan wood extract at the concentration of 4 times of the MIC value in the homemade chili paste. The tests for microbiological quality, moisture content and physiological of chili paste were determined every month for three months.

### ***Free radical scavenging activity determining by 1, 1-Diphenyl 2 picrylhydrazyl (DPPH) method***

Antioxidant activity of the sappan wood extracts and standard solutions (ascorbic acid and trolox) were determined base on the radical scavenging ability in reacting with a stable DPPH free radical.<sup>11</sup> The amount of 100 µl of the extracts (at concentrations of 5 to 100 µg/ml) or a standard was mixed with 200 µl of DPPH 0.2 mM in absolute methanol solution. After incubation at room temperature for 30 minutes in the dark, the absorbance of each solution was measured at 515 nm using a spectrophotometer and a corresponding blank readings were also taken. The DPPH scavenging capacity of sappan wood extracts and standard solutions were expressed as percentage of inhibition (% inhibition) and were calculated by the following equation:

$$\% \text{ Inhibition} = \{1 - (\text{Ab}_{515} \text{ sample} / \text{Ab}_{515} \text{ control})\} \times 100$$

Percentage of fall in absorbance against concentration was plotted and extrapolated to find the inhibition concentration of sample at 50 % fall in absorbance of DPPH (IC<sub>50</sub> value).

### ***Statistical analysis***

The data were analyzed by SPSS 13.0 software program (SPSS Inc. USA.). All data were presented as mean ± standard deviation. The mean values were analyzed using one-way analysis of variance (one-way ANOVA); Tukey multiple comparison was used to approve a significant difference between the means. In case of 2 groups of samples, independent sample T-test was used to approve a significant difference between the means. The significant difference between the means was set at the level of *p*-values < 0.05.

## RESULTS

MIC, MBC and MFC of sappan wood extracts were showed in Table 1. Sappan wood extract 1 showed antibacterial activity at the concentration of 250 µg/ml against *E. coli*, 125 µg/ml against *S. aureus* and 500 µg/ml against *S. typhimurium* which were higher antibacterial activity than the other three extracts and also with good water solubility. The antibacterial activity of sappan wood extract was arranged in order from the highest

of preservation showed that the total aerobic count of CP was higher than 4P (chili paste packed in plastic utensils adding with sappan wood extract at the concentration of 4 times of the MIC value) (39.28 %). During the first nine months of preservation showed that the total aerobic count of CP was higher than 8P (chili paste packed in plastic utensils adding with sappan wood extract at the concentration of 8 times of the MIC value) (42.19 %) (significantly different at the 0.05 level).

**Table 1.** Antimicrobial activities of sappan wood extracts showed as MIC (µg/ml)

Sample	<i>E. coli</i>		<i>S. aureus</i>		<i>S. typhimurium</i>		<i>C. albicans</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Sappan 1	250	500	125	250	500	500	>16,000	>16,000
Sappan 2	250	500	250	500	1,000	2,000	8,000	8,000
Sappan 3	250	500	250	500	500	500	8,000	8,000
Sappan 4	4,000	4,000	1,000	4,000	8,000	8,000	>16,000	>16,000
Chloramphenicol	15.625	31.25	15.625	15.625	3.9	15.625	-	-
Ketoconazole	-*	-	-	-	-	-	0.156	-

\*- = no activity

Sappan wood extract 1 was added to the chili paste produced by the Cooperative, Pathumthani province, at the concentration of 2, 4 and 8 times of the selected maximum MIC (500 µg/ml).

### Remarks for the abbreviations :-

CP = control packed in plastic utensils

2P = chili paste adding with sappan wood extract at the concentration of 2 times of the MIC value (packed in plastic utensils)

4P = chili paste adding with sappan wood extract at the concentration of 4 times of the MIC value (packed in plastic utensils)

8P = chili paste adding with sappan wood extract at the concentration of 8 times of the MIC value (packed in plastic utensils)

CG = control packed in glass utensils

2G = chili paste adding with sappan wood extract at the concentration of 2 times of the MIC value (packed in glass utensils)

4G = chili paste adding with sappan wood extract at the concentration of 4 times of the MIC value (packed in glass utensils)

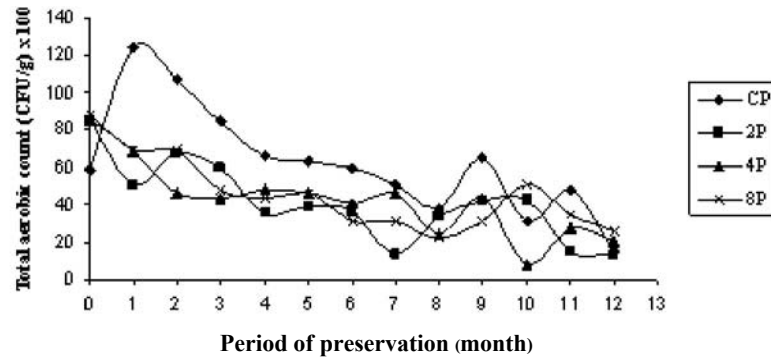
8G = chili paste adding with sappan wood extract at the concentration of 8 times of the MIC value (packed in glass utensils)

as follows; sappan wood extract 1 show higher antibacterial activity than sappan wood extracts 3, 2 and 4, respectively. There fore, the sappan wood extract 1 was selected for the study of food preservative ability in chili paste

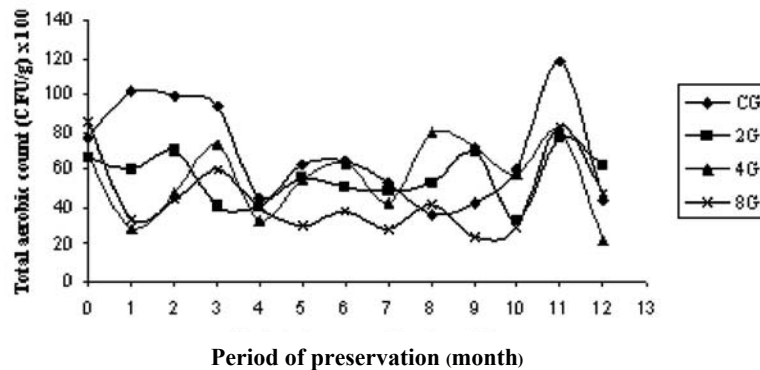
The results of total aerobic plate count (CFU/g) of each chili paste sample which packed in plastic utensils at the period of 12 month preservation were showed in Figure 1. During the first seven months of preservation showed that the total aerobic count of CP (control packed in plastic utensils) was significantly higher than 2P (chili paste packed in plastic utensils adding with sappan wood extract at the concentration of 2 times of the MIC value) (46.13%). During the first six months

During the first six months of preservation showed that total aerobic count of CG was higher than 2G (28.41%). During the first five months of preservation showed that total aerobic count of CG was higher than 4G (37.16%). During the first seven months of preservation showed that total aerobic count of CG was higher than 8G (44.26%) (Figure 2).

Total aerobic count of chili paste at the concentration of 2, 4 or 8 times of the MIC value (plastic and glass utensils) were very variable. Comparing the total aerobic count (CFU/g) of the chili paste between plastic package and glass package was in the variation in total aerobic count of two packages.



**Figure 1.** Total aerobic count (CFU/g) of each chili paste in plastic package at the period of 12 month preservation.



**Figure 2.** Total aerobic count (CFU/g) of each chili paste in glass package at the period of 12 month preservation.

The results of total fungal plate count of each chili paste sample which was packed in plastic utensils at the period of 12 month preservation showed in Figure 3. Total fungal count (CFU/g) of CP was higher than 2P (32.65%) after one month of preservation and higher than 4P and 8P (62.42% and 67.53%, respectively) at 1-2 months of preservation. At 3 month of preservation, the total fungal count of CP, 2P, 4P and 8P were not significantly different.

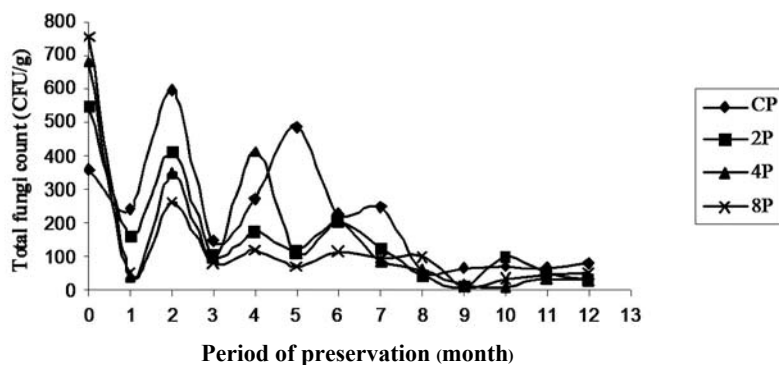
In Figure 4, the results showed that the total fungal count (CFU/g) of CG was lower than 2G and 8G and not significantly different with 4G at one month preservation.

It could not be concluded that sappan wood extract could inhibit fungal in chili paste. Therefore, the study of food preservative

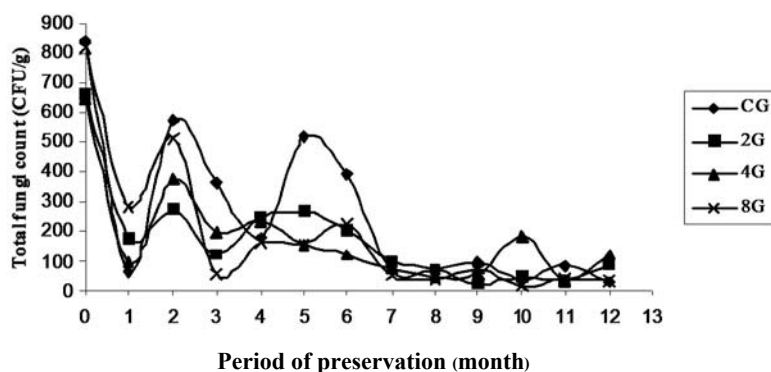
ability of the sappan wood extract in chili paste was repeated.

The MPN coliforms and *E. coli* test showed that MPN coliforms and *E. coli* of all samples were less than 3.0 MPN/g. There were no growth of *S. aureus*, *B. cereus*, *Clostridium perfringens* and *Salmonella* through the period of preservation in all samples.

The moisture of chili paste calculated as the percent of weight loss after drying every month for one year showed that the chili paste moisture content ranged from 45.70 to 57.80%. The smell and color observations of chili paste showed that all the samples were not changed in smell and color through the period of 12 month preservation. All chili paste samples were found to exude their oil after 8 months of preservation.



**Figure 3.** Total fungal count (CFU/g) of each chili paste in plastic package at the period of 12 month preservation.



**Figure 4.** Total fungal count (CFU/g) of each chili paste in glass package at the period of 12 month preservation.

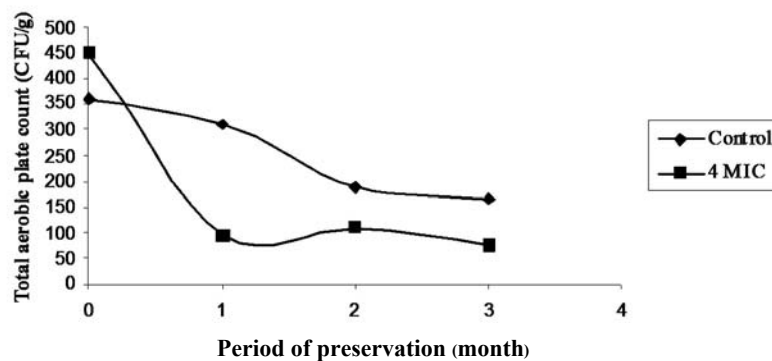
The study of food preservative ability of the sappan wood extract in chili paste was confirmed by adding sappan wood extract at the concentration of 4 times of the MIC value in the homemade chili paste and tested for the microbiological quality, moisture content and physiological appearance of chili paste. The total aerobic count (CFU/g) of chili paste at 4 times of the MIC value (repeated) decreased and was less than control (55.34%) through the period of 3 month preservation as showed in Figure 5.

The total fungal count (CFU/g) of control and chili paste at the concentration of 4 MIC value (repeated) were not significantly difference through the period of 3 month preservation (Figure 6).

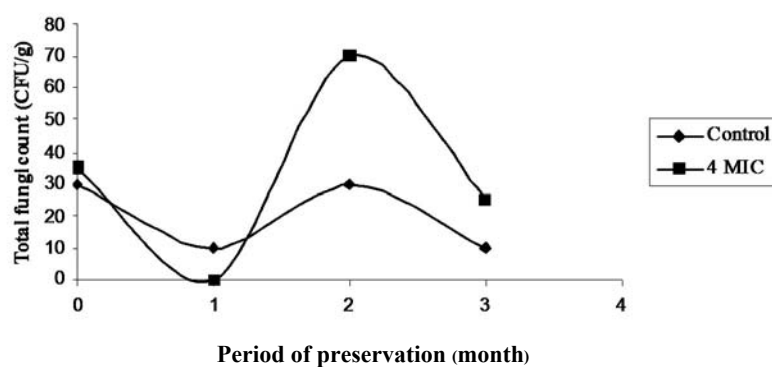
The MPN coliforms and *E. coli* test showed that MPN coliforms and *E. coli* of all samples (repeated) were less than 3.0 MPN/g. There were no growth of *S. aureus*, *B. cereus*, *Clostridium perfringens* and *Salmonella* through the period of 3 month preservation in all samples.

The moisture content of chili paste samples was between 55.66-60.76%. The characteristic smell and color observation of the chili paste showed that all chilli paste samples (repeated) did not changed through the period of 3 month preservation.

Table 2 showed the DPPH radicals scavenging activities of ascorbic acid, trolox, sappan wood extracts 1, 2, 3 and 4.



**Figure 5.** Total aerobic count (CFU/g) of chili paste sample at the period of 3 month preservation.



**Figure 6.** Total fungal count (CFU/g) of chili paste sample at the period of 3 month preservation.

**Table 2.** IC<sub>50</sub> value of ascorbic acid, trolox, sappan wood extracts 1, 2, 3 and 4 on DPPH radicals scavenging activity

Substrate	IC <sub>50</sub> (µg/ml)
Ascorbic acid	17.11
Trolox	24.27
Sappan wood extract 1	23.19
Sappan wood extract 2	38.30
Sappan wood extract 3	29.82
Sappan wood extract 4	71.54

## DISCUSSIONS

The antimicrobial activities of sappan wood extract 1, 2, 3 and 4 were similar to the previous reports.<sup>7, 12, 13</sup> Sappan wood extract 1 had good antibacterial activity which was more than sappan wood extracts 3, 2 and 4, respectively. All sappan wood extracts had low antifungal activity. The difference in antimicrobial activity of sappan wood extract was due to the variation of the chemical composition even in the same species, harvest times and areas of growth and also extraction and drying method.

Total aerobic count of chili paste at the concentration of 2, 4 or 8 times of the MIC value (plastic and glass utensils) was very variable, so it could not be concluded that which concentration at the concentration of 2, 4 or 8 times of the MIC value would be appropriate to be used as preservative. However, at the concentration of 2 time of the MIC value found the bacteria inhibition growth in chili paste until 7 months (in plastic utensils) and 6 months (in glass utensils). Therefore, the sappan wood extract at the concentration of 2 times of the MIC value could be used as preservative in chili paste.

Comparing the total aerobic count of the chili paste between plastic package and glass package was showed as the variation in total aerobic count. It could not be concluded that which package had the better food preservative property.

The results showed that sappan wood extract at the concentration of 2 times of the MIC value could inhibit fungal in the chili paste packed in plastic utensils up to 1 month. Sappan wood extract at the concentration of 4 and 8 times of the MIC value could inhibit fungal up to 2 months. The results of total fungal plate count (CFU/g) of each chili paste sample packed in glass utensils at the period of 12 month preservation were showed in Figure 4. The results showed that sappan wood extract could not inhibit fungal growth in chili paste. It could not be concluded that sappan wood extract could inhibit fungal in chili paste. Therefore, the study of food preservative ability of the sappan wood extract in chili paste was repeated.

The total aerobic plate count and total fungal count (CFU/g) of samples (repeated) at the period of 3 month preservation were showed in Figure 5 and 6, respectively. These results confirmed that sappan wood extract could inhibit bacteria but could not inhibit fungal in chilli paste. So, sappan wood extract could be used as preservative in food for inhibiting bacteria within 6 months.

In this study, free radical scavenging activity of sappan wood extracts was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. Sappan wood extracts had good antioxidants activities; ascorbic acid was more potent than sappan wood extract 1, trolox, sappan wood extract 3, sappan wood extract 2 and sappan wood extract 4, respectively (Table 2). Sappan wood extracts are good antioxidants agreed with those previous reports.<sup>14,15</sup> The observed antioxidant activity of the heartwood might be due to the presence of brazilin, flavonoids and phenolic compounds present in it.<sup>14</sup> Sappan wood extract is appropriate to increase the value of products and could be useful for health.

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