The 4th Thailand International Symposium on Natural History Museums:
Era of Ecosystem Restoration, 185–195.

Evaluation of *Launaea sarmentosa* (Willd.) Sch. Bip. ex Kuntze crude extracts

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Article History
Received: 9 January 2024
Accepted: 6 August 2024
Published Online: 23 August 2024

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Abstract

Antioxidant agents play a pivotal role in the treatment of diseases and wound healing. The biennial plant, *Launaea sarmentosa* (Willd.) Sch. Bip. ex Kuntze, from the Asteraceae, possesses essential phenolic compounds such as flavonoids and polyphenols. Historically, this plant has demonstrated significant healing properties for various wounds, including those caused by itching, infection, blisters, and conditions like herpes zoster. Given its potential therapeutic benefits, this study sought to evaluate the crude extracts of *L. sarmentosa* for their antioxidant, antibacterial, and anti-inflammatory properties. Leaves harvested during both rainy and summer seasons from Phuket Province, Thailand were subjected to extraction using five solvents: hexane, ethyl acetate, ethanol, methanol, and distilled water. Total phenolics analysis indicated that the distilled water solvent yielded the highest concentrations, with values of 55.689±0.452 mg GAE/g and 95.740±0.484 mg GAE/g dry weight for the rainy and summer samples, respectively. Similarly, total flavonoid contents were highest in these extracts, with values of 205.566±0.782 mg QUE/g and 266.550±4.059 mg QUE/g dry weight. Antioxidant activities were evaluated using the DPPH scavenging test, with the methanol extracts displaying the most potent activity, possessing IC_{50} values of 0.057±1.355 mg/mL (rainy season) and 0.050±3.044 mg/mL (summer season). These results underscore the potential of *L. sarmentosa* as a key ingredient for the formulation of hydrogel wound healing patches.

Keywords
Antioxidant, *Launaea sarmentosa*, seasons, TFC, TPC

Citation. Ramangthong, W., N. Chanchula and P. Na Nakorn. 2024. Evaluation of *Launaea sarmentosa* (Willd.) Sch. Bip. ex Kuntze crude extracts. In *The 4th Thailand International Symposium on Natural History Museums: Era of Ecosystem Restoration* on 21–22 December 2023 at Rama 9 Museum Thailand: 185–195 pp.

Introduction

The cost of treating wounds caused by microbial infections and inflammatory tissue is a significant burden on the healthcare system, as seen in various countries in the world such as in the United Kingdom, medical expenses for wound care account for 3% of total medical expenses. In the United States, wound care costs reach as high as $20 billion yearly (Frykberg and Banks, 2015). Thailand also faces challenges, with bloodstream infections being the third leading cause of death in 2023, and tissue, wound, and skin infections ranking among the top ten causes of outpatient death (Ministry of Public Health, 2023). The wound healing process typically takes around 12 weeks, and if it takes too long, it can result in chronic wounds that may lead to infection and even death (Gurtner *et al.,* 2008). Therefore, promoting the wound healing process is critically for maintaining tissue integrity after injury (Boateng *et al.,* 2008).

Throughout history, humans have utilized plants for medicinal purposes, and plants produce compounds known as phytochemicals, which have various properties. Phytochemicals are organic compounds consisting of carbon atoms linked to hydrogen, oxygen, nitrogen, or other carbon atoms. Secondary phytochemicals, such as polyphenol compounds and flavonoid compounds, possess antioxidant abilities and can exhibit biological effects such as anti-microglial, anti-inflammatory abilities, and many others from alternating single bonds of cogitating bonding on the ring. (Santos-Sánchez *et al.,* 2019)

One interesting plant is *L. sarmentosa* (Willd.) Sch. Bip. ex Kuntze in the Asteraceae family. It is an annual crops plant with a short life cycle, tongue-like leaves, wavy leaf edges, curved leaf tips, and green leaf blades measuring approximately 20–25 cm in length. *Launaea sarmentosa* produces yellow inflorescences with tightly clustered petals and can commonly be found near beaches at sea. *Launaea sarmentosa* has healing properties, with fresh leaves being used to treat various skin wounds, including itching wounds, fresh wounds, rotting wounds, purulent inflamed wounds, blisters, herpes, shingles, and others. They are famous for their ability to treat burning pain, reduce swelling, and alleviate inflammation (Prapasanobol and Kaewsrasan, 2012). Previous research has identified three groups of important substances in *L. sarmentosa*, namely steroids, flavonoids, and glycosides, which contribute to their wound healing and anti-inflammatory effects (SubUdompol and Phuwaplaisirisal, 2008; Raju *et al.,* 2014; Nguyen *et al*., 2020; Shubhangi *et al*., 2022). Considering the pharmacological properties of *L. sarmentosa*, this research aimed to analyze their extracts and evaluate various biological properties and activities, including the total phenolic contents, total flavonoid contents, and antioxidant activity.

Materials and methods

Preparation of *Launaea sarmentosa*

The fresh leaves for the *L. sarmentosa* were collected during the rainy and summer seasons from farmers in Phuket province, Southern Thailand. These samples were then washed and dried in a hot air oven at 50 °C. After that, dry samples were grounded. Then, the samples were stored in opaque containers and protected from moisture exposure before the following methods. Moreover, the samples were tested for contaminations, specifically, the four groups of insecticide contaminants, namely: organophosphates group, carbamates group, organochlorines group, and pyrethroids group. They were examined by using the GPO-TM/1 test kit and GPO-TM/2 test kit. These test kits are the principle of the TLC method for separating and detecting different types of insecticide chemicals.

Extraction of *Launaea sarmentosa*

This study was conducted by extracting the leaves of *L. sarmentosa* from two seasons (rainy and summer) by maceration extraction and using 5 different solvents: hexane, ethyl acetate, ethanol, methanol, and distilled water. Dried samples of *L. sarmentosa* were soaked in each solvent with ratio of 1:10 (w/v) and left at room temperature for three days. Then the mixture was coarsely filtered using a filter cloth. Then, it was finely filtered using a vacuum filter by Whatman's filter paper No. 2. The filtrates were evaporated by a rotary evaporator to

obtain crude extracts of *L. sarmentosa.* Moreover, in case of distilled water crude extract, it was then freeze dried after evaporation before storage. Therefore, each solvent crude extracts of the rainy and summer seasons were obtained. These samples were stored in amber glass bottles at 4 °C, except distilled water crude extract at -4 °C, for further studies.

Determination of total phenolic contents by Folin-Ciocalteu method (Wolfe *et al.*, 2003)

Pipette a sample stock solution of 1 mg/mL crude extracts 125 µL was added to a test tube with 500 µL distilled water and Folin-Ciocalteu reagent 125 µL, then mixed well with vortex, and left for 6 min. Then, 7% sodium carbonate solution of 1250 µL was added and mixed well. It was left at room temperature for 90 min for color development, then 1000 µL of distilled water was added and measured of absorbance by UV-VIS Spectrophotometer. At a wavelength of 760 nm, the values obtained were compared with the standard curve of Gallic acid. The total phenolic contents were determined as mg of gallic acid equivalent per gram of the crude extract (mg GAE/g crude extract) from the following equation:

$$TPC = \frac{(C)\,(V)}{M}$$

By substituting the following equation:

TPC = total phenolic contents (mgGAE/g extract)
C = concentration calculated from the standard curve (mg/mL)
V = volume of solution of prepared sample (mL)
M = dry plant weight (g) of the crude extract from which the sample solution was prepared

Determination of total flavonoid contents (Wolfe *et al.*, 2003)

Pipette a sample stock solution of 1 mg/mL crude extracts 250 µL was added to a test tube with 1250 µL distilled water and 5% sodium nitrite 75 µL, then mixed well with vortex, and collected in the dark for 5 min. Then, 10% aluminum chloride of 150 µL was added, mixed well, and collected in the dark for 5 min. 1 M sodium hydroxide 500 µL and distilled water 275 µL were then added. Then, it was mixed well and collected in the dark for 5 min for color development. Measured absorbance by UV-VIS Spectrophotometer. At a wavelength of 510 nm, the values obtained were compared with the standard curve of Quercetin. The total flavonoid contents were determined as mg of Quercetin equivalent per gram of the crude extract (mg QUE/g crude extract) from the following equation:

$$TPC = \frac{(C)\,(V)}{M}$$

By substituting the following equation:

TFC = total flavonoid contents (mgQUE/g extract)
C = concentration calculated from the standard curve (mg/mL)
V = volume of solution of prepared sample (mL)
M = dry plant weight (g) of the crude extract from which the sample solution was prepared.

Determination of antioxidant activity by 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity assay (Tosun *et al.*, 2009)

The concentration of the crude extracts was diluted to concentrations of 1, 10, 50, and 100 µg/mL. Then, a pipet of 100µL of each concentration sample was put into a 96-well plate, 100µL of 0.08 mM DPPH solution was added, and mixed well with a vortex. After that, it was kept in the dark for 30 min for color development. The absorbance value was measured with a microplate reader at a wavelength of 515 nm. The obtained value was used to calculate the DPPH inhibition activity (%) according to the following equation. Then, it was plotted in the graph equation between % Inhibition (y) and the concentration of crude extracts (mg/mL) (x) to quantify the antioxidant value at 50% or IC_{50}.

$$\text{DPPH inhibition activity (\%)} = \left(\frac{AB\text{-}AA}{AB} \right) \text{x } 100$$

By substituting the following equation:

AA = Absorbance value of the sample + DPPH

AB = Absorbance value of the DPPH solution

Statistical analysis

This research was Experimented with at least three times. Analysis the results and present the data in the form of mean, variance deviation of the mean, and standard deviation (mean±SD) by Microsoft Excel and GraphPad Prism programs. Analysis ANOVA at 99% confidence by IBM SPSS Statistics program. Analysis pairwise comparison using Tukey's test by the JASP program.

Results and discussion

Preparation of *Launaea sarmentosa* samples

Analyses of insecticidal contaminants were conducted the samples with GPO test kits of four groups insecticidal contaminants (18 standard substant), namely: clofenvinphos, chlorpyrifos, dichlorvos, dicrotophos, monocrotophos, profenofos, bendiocarb, carbaryl, carbofuran, methomyl, DDT, endrin, endosulfan, cypermethrin, permethrin, deltamethrin, herbicides, and 2,4-D. All samples were tested and revealed that there were no insecticidal contaminants found, as shown in Table 1 Therefore, the samples were utilized in this research, revealed the high purity, and the analysis results would not be affected by any contaminants (Shakir *et al.*, 2018). Moreover, the extracts are safe for the further utilization with human.

Extraction of *Launaea sarmentosa*

Extraction of *L. sarmentosa* leaves was accomplished by using five different solvents, namely: hexane, ethyl acetate, ethanol, methanol, and distilled water. It was found that the % yield of crude extracts from the leaves of *L. sarmentosa* in the rainy season were 8.982%, 8.956%, 8.690%, 10.121%, and 4.040%, respectively. On the other hand, the samples in the summer season were 10.268%, 10.245%, 10.196%, 11.993%, and 5.651%, respectively, as shown in Table 2. The results of this experiment indicated that polar solvents, especially, methanol, are it is about solubility of chemical compounds in plants in extracting the crude extracts from the leaves. However, in case of distilled water, it seemed to be obtained at lowest % yield, but the crude extract samples were freeze dried afterward; therefore, getting the lowest value. This is consistent with previous research that suggests that plants contain important substances in the phenolic and flavonoid groups, which have the property of dissolving well in polar solvents. As a result, using medium-high polarity solvents tends to yield a higher amount of extract compared to less polar or non-polar solvents (Pinelo *et al.,* 2004).

Table 1. The result of insecticidal contaminants detection in *L. sarmentosa* samples.

Substances group	Standard substance	Results
Organophosphates group	Chlorfenvinphos	-
	Chlorpyrifos	-
	Dichlorvos	-
	Dicrotophos	-
	Monocrotophos	-
	Profenofos	-
Carbamates group	Bendiocarb	-
	Carbaryl	-
	Carbofuran	-
	Methomyl	-
Organochlorines group	DDT	-
	Endrin	-
	Endosulfan	-
Pyrethroids group	Cypermethrin	-
	Permethrin	-
	Deltamethrin	-
	Herbicides	-
	2,4-D	-

Note: This table shows the analysis of insecticides in the rainy and summer of *L. Sarmentosa.* (+) = Found, (-) = Not found

Table 2. The percent yield of *L. sarmentosa* crude extracts collected in 2 different seasons.

Solvent	%yield	
	Rainy	Summer
Hexane	8.972	10.268
Ethyl acetate	8.956	10.245
Ethanol	8.690	10.196
Methanol	10.121	11.993
Distilled water	4.040	5.651

Total phenolic contents determination

Analysis of total phenolic contents from the crude extracts of rainy and summer season leaves using five types of solvents (hexane, ethyl acetate, ethanol, methanol and distilled water) are shown in Table 3. It was interesting that in the rainy season, the extracts obtained from hexane and ethyl acetate, as well as ethanol solvents, did not show significant differences in total phenolic contents (TPC). However, in the summer season, it was observed that all solvents showed significant differences. Whereas methanol and distilled water extracts revealed differences and high TPC that also related to the previous % yield results. This

indicated that the crude extracts obtained from methanol and distilled water, which are highly polar solvents, exhibited the highest number of phenolic compounds even though in both seasons. This is due to the formation of hydrogen bonds in polar solutions, which affects the ability of phenolic compounds to donate hydrogen atoms and consequently influences the yield of these important substances (Dai and Mumper, 2010). When considering the season, the statistical analysis showed significant differences TPC of each solvent between the rainy and summer seasons, Vanhakylä and Salminen (2023) identifying the different amount of phenolic contents and variation depending on the seasonal of the plants. In this case, the summer season caused a high salt stress level in *L. sarmentosa* resulting in an increase in oxidative stress in the plants, which was revealed through the accumulation of malondialdehyde and hydrogen peroxide content. Therefore, total phenolic content and the activities of antioxidant enzymes such as catalase, peroxidase, and superoxide dismutase in *L. sarmentosa* were increased (Tran *et al.,* 2024).

Table 3. Total phenolic contents (mg GAE/g crude extract) in *L. sarmentosa* crude extracts collected in 2 different seasons.

TPC	**Solvents**				
Samples	**Hexane**[1/]	**Ethyl acetate**[1/]	**Ethanol**[1/]	**Methanol**[1/]	**Distilled water**[1/]
Rainy	21.093±0.342^{d}	23.261±0.341^{cd}	26.908±2.821^{c}	43.072±0.744^{b}	***55.689±0.452^{a}***
Summer	44.163±0.558^{e}	47.386±0.838^{d}	51.738±0.484^{c}	88.971±1.279^{b}	***95.740±0.484^{a}***
	**	**	**	**	**

Note: The numbers in the table show the average ± standard deviation.
[1/] Values directed with different characters in column, indicating that there are differences at 99% confidence when comparing by Tukey's test, NS = not statistical differences and ** = Statistical differences at 99% confidence
a-d = Statistical differences at 99% confidence with different characters in row

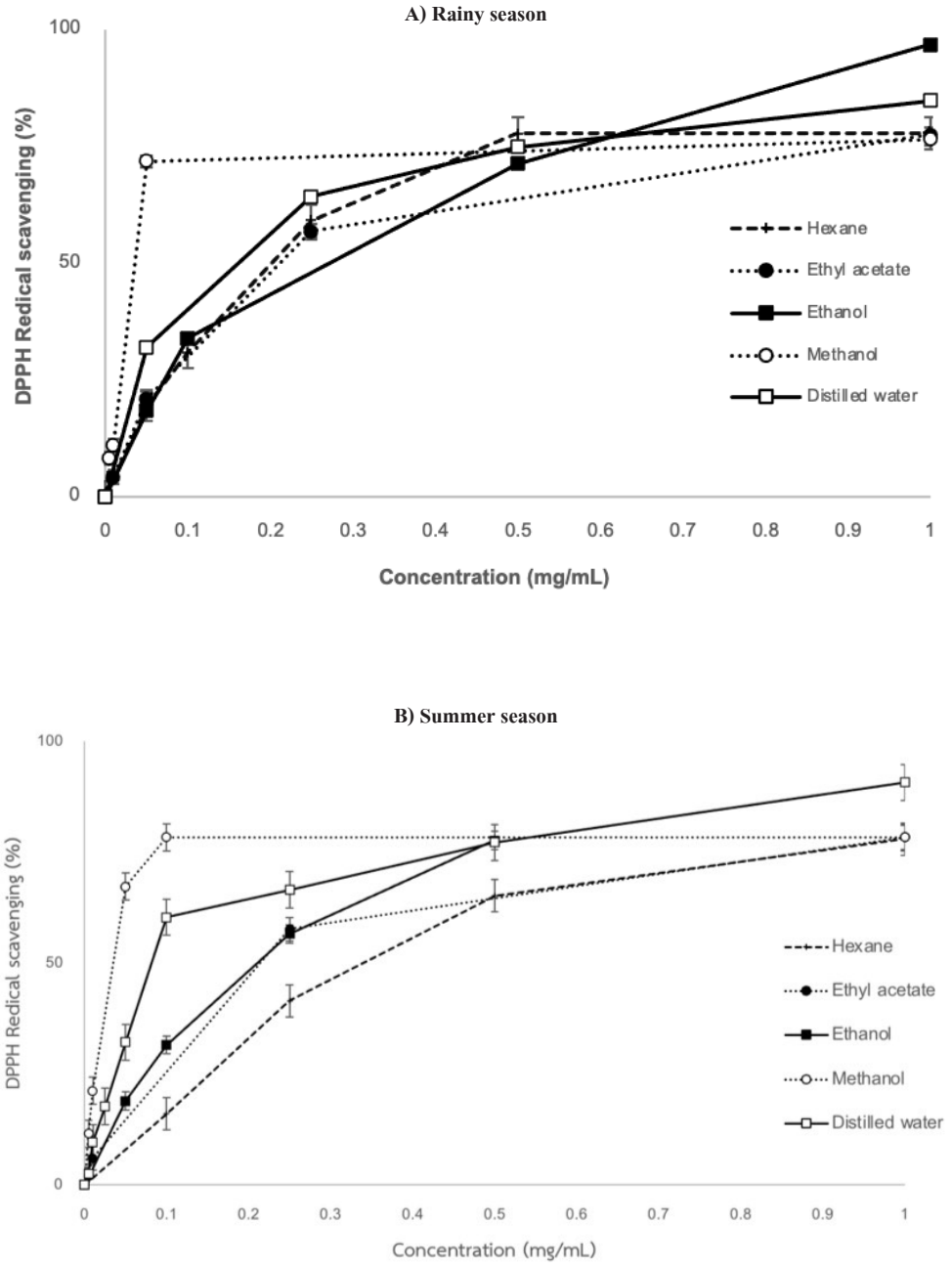

Figure 1. DPPH radical scavenging (IC_{50}) of *L. sarmentosa* crude extracts collected in 2 different seasons (A-B) with 5 different solvents.

Total flavonoid contents determination

Analysis of total flavonoid contents (TFC) are shown in Table 4. The results of statistical analysis showed that in the rainy season, the results of the solvents ethyl acetate and ethanol were not significantly different, as in the summer. In addition, when considering the season, it

was found that only the hexane solvent extract showed no significant difference in the amount of flavonoid compounds. It is interesting to note that the results of the experiment indicated that the plant samples collected during the summer season had higher amounts of extracts and important substances compared to those collected during the rainy season. This can be attributed to the lack of water during the summer, which forces stress on plants. In response to this stress, plants adapt by activating secondary metabolism mechanisms, leading to an increase in secondary metabolites such as flavonoids and phenols. This finding is consistent with previous research conducted by Sara *et al.* (2016), which also demonstrated that a reduced amount of water can result in an increased production of flavonoids and phenols in plants. The crude extract from methanol and distilled water depicted the high amount of both TPC and TFC related to the amount of crude extract (%yield).

Table 4. Total flavonoid contents in *L. sarmentosa* crude extracts collected in 2 different seasons.

TFC	**Solvents**				
Samples	**Hexane**[1/]	**Ethyl acetate**[1/]	**Ethanol**[1/]	**Methanol**[1/]	**Distilled water**[1/]
Rainy	68.673±3.411[d]	64.453±1.565[c]	167.615±0.782[c]	181.621±1.355[b]	***205.566±0.782***[a]
Summer	83.773±3.658[d]	211.482±2.684[c]	218.512±2.029[c]	233.743±3.044[b]	***266.550±4.059***[a]
	NS	**	**	**	**

Note: The numbers in the table show the average ± standard deviation.
[1/] Values directed with different characters in column, indicating that there are differences at 99% confidence when comparing by Tukey's test, NS = not statistical differences and ** = Statistical differences at 99% confidence
a-d = Statistical differences at 99% confidence with different characters in row

Antioxidant activity of crude extracts

Analyses of the antioxidant activities of those five crude extracts from two different seasons were presented in DPPH radical scavenging of IC_{50} value (Table 5 and Picture 1). It was shown that in the rainy and summer season, hexane, ethyl acetate and ethanol extracts were not significantly different; whereas, one of methanol and distilled water extract revealed a different and better antioxidant activity compared to the others that related to the results of TFC and TPC. The extracts from polarity solvents exhibited higher antioxidant activity compared to those extracted from non-polarity solvents. This can be attributed to the presence of phenolic compounds and flavonoids which are important substances in medicinal plants. These compounds are more soluble in polarity solvents than in non-polarity solvents. This is consistent with previous research indicating that the amount of important substances found in plants is related to their antioxidant activity. (Phowichit *et al.,* 2019). It is interesting to note that in this experiment, samples extracted from distilled water in the rainy season showed lower antioxidant activity compared to methanol, which has lower polarity. It was shown that the antioxidant activity of crude extract in methanol solvent does not only come from phenolic compounds and flavonoids, but there may be other important substances that the methanol solvent in the rainy season can extract. As a result, the extract had a better antioxidant activity. The summer season revealed the better characteristics (TPC, TFC and IC_{50}) than the one from rainy season, certainly.

Table 5. IC_{50} in *L. sarmentosa* crude extracts collected in 2 different seasons.

IC_{50} (mg/mL)	Solvents				
Samples	**Hexane**[1/]	**Ethyl acetate**[1/]	**Ethanol**[1/]	**Methanol**[1/]	**Distilled water**[1/]
Rainy	0.278±3.411^{c}	0.206±1.565^{cd}	0.198±0.782^{d}	***0.057±1.355^{a}***	0.151±0.782^{b}
Summer	0.331±3.658^{c}	0.149±2.684^{b}	0.198±2.029^{b}	***0.050±3.044^{a}***	***0.078±4.059^{a}***
	NS	**NS**	**NS**	**	**

Note: The numbers in the table show the average ± standard deviation.
[1/] Values directed with different characters in column, indicating that there are differences at 99% confidence when comparing by Tukey's test, NS = not statistical differences and ** = Statistical differences at 99% confidence
a-d = Statistical differences at 99% confidence with different characters in row

Summary

Plants are a source of medicine, treatment, and disease management. These properties come from compounds that plants produce naturally, called phytochemicals. Some secondary phytochemicals have antioxidant abilities and can have a variety of pharmacological effects. The *L. sarmentosa* is classified as a native plant of Thailand that has properties to heal wounds very well. Previous research has found that *L. sarmentosa* has the ability to heal wounds and reduce inflammation, because it contain 3 groups of important substances, including steroids, the flavonoid group, and the glycoside group (SubUdompol and Phuwaplaisirisal. 2008). In this research, it was found that summer plant samples had high % yielded of extracts, total phenolic contents, total flavonoid contents, and better antioxidant activity than plant samples in the rainy season, because in the summer, the plants receive less water causing to plants to exposed to unfavorable environmental factors, resulting in stress. Plants therefore create mechanisms to protect themselves against the secondary metabolism mechanisms, increasing secondary substances in plants (Prinsloo and Nogemane, 2018). In addition, from the results of TPC and TFC, it was found that the suitable solvents for extraction to obtain such important substances were polar solvents like distilled water and methanol, which produced the highest amounts of active substances. Meanwhile, the low polarity solvent, hexane, produced the least number of active substances in both seasons. This emphasizes that plants that contain substances in the phenolic and flavonoid groups had the property of being able to dissolve well in polar solvents. From the results of the DPPH antioxidant activity experiment, it was found that in the rainy season, methanol extracts provided better antioxidant activity than extracts with higher polarity, such as distilled water. This is inconsistent between the amount of important substances obtained and their antioxidant activity. Therefore, it is possible that the antioxidant activity of some extracts may not only come from phenolic compounds and flavonoids, but may have other important substances involved.

From all results, it can be concluded that *L. sarmentosa* from methanol and distilled water solvent are suitable for further development, because of highly important substances namely: phenolic compound, flavonoids and good antioxidant activity, which could be used reduce Inflammation in wound or other uses.

Acknowledgements

The authors gratefully acknowledge the financial support provided by Faculty of Science and Technology, Contract No. SciGR20/2565. Moreover, partially supported by NRCT grant with Assistant Prof. Dr. Nattapong Chanchula.

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