International Journal of Agricultural Technology 2023 Vol. 19(1):277-290
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Phytochemical screening, antioxidant activity and total phenolic content of methanolic extract of Phak Wan Ton (*Crotalaria medicaginea* Lam.)

Thuion, T., Poeaim, S.* and Poeaim, A.

Department of Biology, School of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520, Thailand.

Thuion, T., Poeaim, S. and Poeaim, A. (2023). Phytochemical screening, antioxidant activity and total phenolic content of methanolic extract of Phak Wan Ton (*Crotalaria medicaginea* Lam.). International Journal of Agricultural Technology. 19(1):277-290.

Abstract The chlorophyll content of the methanolic extract of *Crotalaria medicaginea* without chlorophyll removal ranged from 0.50±0.03 to 1.24±0.06 mg/g extract, and with chlorophyll removal ranged from 0.09±0.01 to 0.18±0.00 mg/g extract. The highest total phenolic content using the Folin-Ciocalteu method was presented in the leaves extract without chlorophyll removal as 48.33±0.51 mg GEA/g extract. Moreover, the leaves extract without chlorophyll removal showed the lowest antioxidant activity with 50% inhibitory concentration (IC_{50}) in DPPH radical scavenging assay at 344.76 µg/ml. Besides, the root extract exhibited rich potential for gram-positive bacteria, including *Bacillus cereus, Bacillus subtilis, Kocuria rhizophila, Staphylococcus aureus* and *Staphylococcus epidermidis.* The overall results showed that the extracts without chlorophyll removal had more efficiency than those with chlorophyll removal. Hence, the phytochemical screening was investigated only in the methanolic extract without chlorophyll removal. All extracts found alkaloids and coumarins, while tannins were found only in roots and did not display saponins. The current study suggested that the extracts of *C. medicaginea* Lam. gave the potential source of natural bioactive compounds.

Keywords: Antioxidant, Chlorophyll removal, *Crotalaria medicaginea*, Phytochemical, Total phenolic content

Introduction

Medicinal plants are important in various ailments due to the bioactive compounds hidden in those plants, such as alkaloids, flavonoids, coumarins and tannins. These are mostly phenolic compounds, essential bioactive compounds in plants presented as antimicrobial, antioxidant, anticarcinogen and anti-inflammatory (Elangovan *et al*., 1994; Stavric, 1994; Ngoci *et al*., 2011). Therefore, drug research is essential to evaluate biological activities and identify and isolate natural bioactive or secondary metabolized compounds from plant materials.

* **Corresponding Author**: Poeaim, S.; **Email**: supattra.poe@kmitl.ac.th

Crotalaria medicaginea Lam. commonly known in Thai as Phak Wan Ton, is a folk medicinal plant belonging to the genus *Crotalaria* and the family Fabaceae. The distribution of this genus has been displayed in India, China and Southeast Asia, such as Malaysia and Thailand (Niyomdham, 1978; Yadava and Vishwakarma, 2014; Ninkaew *et al*., 2018). Most medicinal attributes have been presented in the whole plant, traditionally used as an analeptic for rheumatism and myofascial pain syndrome treatment (Ninkaew *et al*., 2018), impetigo skin, and used as a blood tonic in anemia (Jain *et al*., 2009). Besides, *C. medicaginea* contained bioactive compounds, including alkaloids, phenolics and flavonoids, that highly presented antioxidant and antibacterial activities in vivo and in vitro from ethanolic extract (Devendra *et al*., 2012). In addition, bioactive compounds such as quercitrin, acacetin, isorhamnetin and new allelochemicals have been isolated from methanolic extract of the stems of *C. medicaginea* (Yadava and Vishwakarma, 2014). Those new allelochemicals exhibited antimicrobial activities against various bacteria and inhibited the growth of pathogenic fungi such as *Aspergillus niger*, *Candida albican* and *Mucor indicus*.

Chlorophyll is almost present in green color, found in the chloroplast of the leaves, stems and various parts of any green plants (Kwartiningsih *et al*., 2021). Chlorophyll is an important component for the absorption of sunlight in photosynthesis to provide energy (Hörtensteiner and Kräutler, 2011). Chlorophyll contains anti-inflammatory, antibacterial, antifungal, antioxidant, wound healing, anticarcinogenic activity and detoxification activity (Breinholt *et al*., 1995; Yin and Cheng, 1998; Ferruzzi and Blakeslee, 2007). Hence, chlorophyll is widely used as alternative medicine, supplementary food, natural food colorant, beverage and food industries. The plant leaves contain some pharmacologically active. However, chlorophyll in the crude extract may reduce the interest in products (Phaisan *et al.*, 2020). Chlorophyll can dissolve in a wide range of organic solvents (Phaisan *et al.*, 2020) such as ethanol, methanol, ethyl ester, petroleum ether, acetone and chloroform. Likewise, chlorophyll can be inevitably contained in the processing of plant extraction at various concentrations, presents the dark-greenish color of the extracts and might interfere with the analysis or resulting extract. Therefore, the use of extract was limited, especially in food applications, because the dark-greenish color causes a change in color in food or hides other colors (Namal Senanayake, 2013; Olatunde *et al*., 2018). Additionally, chlorophyll pigments have wavelengths absorption between 400 and 700 nm. There is the measurement range used to indicate biological endpoints. Thus, a greenish color might interfere with in vitro and in vivo bioassay readings on

fluorescence measurements, cause to inaccurate analysis (Denizot and Lang, 1986; DeGraff and Mitchell, 1987).

A previous study reported the ethanolic extracts with dechlorophyllization by sedimentation process had higher antioxidant activity potential than those without dechlorophyllization (Tagrida and Benjakul, 2020). Another method was reported as the effective method for chlorophyll removal from *Chromolaena odorata* using various oils. Palm oil and soybean oil showed high total phenolic and flavonoid content and high efficiency for chlorophyll removal (Phaisan *et al*., 2020). Therefore, the study aimed to investigate the phytochemicals, total phenolic contents, antioxidant and antibacterial activities of *C. medicaginea* with and without chlorophyll removal.

Materials and methods

Extraction of Crotalaria medicaginea

C. medicaginea was collected from Suphanburi province, Thailand. The plants were separated into roots, stems and leaves. These were dried at 40 ℃ and ground to powder. The maceration method was prepared for extraction in one week. The mixture was filtrated and concentrated to obtain the methanolic extract of roots, stems and leaves of *C. medicaginea*.

Chlorophyll removal

The method of chlorophyll removal from the extract has modified the method of Phaisan *et al*. (2020) using palm oil by the liquid-liquid extraction method. The crude extract of the stems and leaves without chlorophyll removal was dissolved in methanol at the ratio of 1:20 (w/v). The mixtures were mixed with palm oil and centrifuged at 5000 rpm for 25 min. The upper phase was collected and concentrated to obtain crude extract with chlorophyll removal.

Determination of chlorophyll content

The stems and leaves were used to determine the chlorophyll content following the method of Olatunde *et al*. (2018). The crude extracts were dissolved with methanol to a concentration of 2 mg/ml, and measured absorbance of wavelengths at 645 and 663 nm using a microplate reader. The chlorophyll a, chlorophyll b and total chlorophyll content were calculated using the below equations: Chlorophyll a (mg/ml) = 12.7 (A_{663}) - 2.69 (A_{645}),

Chlorophyll b (mg/ml) = 22.9 (A_{645}) - 4.68 (A_{663}) and Total chlorophyll (mg/ml) = 20.2 (A_{645}) + 8.02 (A_{663}).

Determination of total phenolic content

Five different extracts of *C. medicaginea* consisted of roots, stems and leaves without chlorophyll removal and stems and leaves with chlorophyll removal. All extracts were estimated with the total phenolic content using the Folin-Ciocalteu method modified from Chantarasaka *et al*. (2022). The reaction was 10% Folin-Ciocalteu reagent, 7.5% sodium carbonate (Na_2CO_3), and gallic acid was used as standard. The total phenolic content was presented as mg of gallic acid equivalent (GAE)/g extract.

Determination of antioxidant activity

All extracts estimated the antioxidant activity using DPPH free radical scavenging assay following the method of Armania *et al*. (2013) with few modifications. The extract concentrations ranged from 125 to 2000 µg/ml. 0.1 mM methanolic DPPH was used as a reaction. The Trolox was used as standard. Methanol was used instead of samples for control. The percentage of DPPH radical scavenging activity was estimated following the below equations, and antioxidant activity with 50% inhibitory concentration (IC_{50}) was examined by GraphPad Prism 5.0 program.

$$\% \text{ DPPH radical scavenging activity} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of a sample or standard.

Evaluation of antibacterial activity

Five different extracts of *C. medicaginea* were evaluated for their antibacterial activity against gram-positive bacteria, including *Bacillus cereus*, *Bacillus subtilis*, *Kocuria rhizophila*, *Staphylococcus aureus* and *Staphylococcus epidermidis* and gram-negative bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa*. The antibacterial activity was estimated using Kirby-Bauer's disc diffusion method, according to the CLSI adopted in 2012. The bacteria were cultured into Mueller- Hinton Broth (MHB). The tested extract concentration was 2 mg/disc. One µg/disc of Gentamicin was

used as a positive control, while methanol and Dimethyl sulfoxide (DMSO) was used as the negative control.

Phytochemical screening

The primary phytochemical activity of methanolic extracts of *C. medicaginea* was determined according to Lordkhem (2016), Sreeprasert (2016), and Thiankathet *et al*. (2019)

Test of alkaloids

Each extract was prepared in 1% hydrochloric acid solution (HCl). The mixtures were filtered and tested with three different reagents: Dragendorff's, Mayer's, and Wagner's. The precipitates of the mixture solution were observed and compared with the colchicine as a standard.

Test of tannins

Each extract was dissolved in distilled water, and 10% sodium hydroxide (NaCl) was added. Three different reagents were used for testing, including gelatin solution, gelatin salt solution, and 1% ferric chloride ($FeCl_3$). The white residues of the mixture solution were observed on the test tubes, which added gelatin solution and gelatin salt solution. The brownish-green or dark blue precipitates were observed on the test tubes, adding 1% $FeCl_3$. Tannic acid was used as a standard.

Test of coumarins

Each extract was dissolved in 50% ethanol and filtered by Whatman paper no. 1. 6M NaOH was added. The dark green or dark yellow color was compared with warfarin as a standard.

Test of saponins

The froth test was examined by dissolving the extracts in distilled water. The mixtures were filtered and shaken. The stable foam was observed for about 30 min. The saponin solution was used as a standard.

Statistical analysis

The information was with the one-way analysis of variance (ANOVA) by using the IBM SPSS Statistics program version 26 (SPSS Inc., USA).). The multiple range tests were compared by Duncan's at the significant differences ($P<0.05$). The GraphPad Prim 5.0 program was used to determine the IC_{50}.

Results

Extraction and chlorophyll removal

The extraction yield of roots was 3.70%, and stems and leaves without chlorophyll removal were 4.02 and 16.96%, respectively. Stems and leaves with chlorophyll removal were 0.33 and 0.44%, respectively. The methanolic extracts of roots showed yellow color and powdered solid. Leaves without chlorophyll removal showed a rather greenish-dark color than stems without chlorophyll removal; both were viscous textures. However, the extract of the leaves with chlorophyll removal showed a slightly greenish color when compared with leaves without chlorophyll removal extracts. Stems with chlorophyll removal showed brown-yellow color; both textures were powdered solid.

Chlorophyll content in methanolic extracts

Chlorophyll a, chlorophyll b, and total chlorophyll content in methanolic of stems and leaves extract without chlorophyll removal and with chlorophyll removal were determined (Table 1). As a result, chlorophyll a, chlorophyll b, and total chlorophyll in the extracts without chlorophyll removal exhibited the highest chlorophyll content, especially in the leaves. However, the chlorophyll content in stems and leaves with chlorophyll removal decreased.

Table 1. Chlorophyll content in methanolic extracts of *C. medicaginea*

Sample	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Roots	nd	nd	nd
Stems without chlorophyll removal	0.35 ±0.02	0.15 ±0.01	0.50 ±0.03
Leaves without chlorophyll removal	0.78 ±0.03	0.46 ±0.03	1.24 ±0.06
Stems with chlorophyll removal	0.03 ±0.01	0.06 ±0.00	0.09 ±0.01
Leaves with chlorophyll removal	0.08 ±0.00	0.10 ±0.00	0.18 ±0.00

nd = not determined

Determination of total phenolic content

The total phenolic content was calculated by a linear equation of $y = 0.0176X$, $R^2 = 0.9993$, a linear calibration curve of gallic acid. The

methanolic extract from leaves without chlorophyll removal and with chlorophyll removal exhibited a high value of total phenolic content. However, the results exhibited the highest total phenolic content in the leaves extracts without chlorophyll removal as 48.33±0.51 mg GEA/g extract. The amount of total phenolic content compared with stems (17.52 and 18.56 mg GEA/g extract) and roots extract (19.62 mg GEA/g extract) showed no significant differences in the extraction (Table 2).

Table 2. Total phenolic content and antioxidant activity in methanolic extracts of *C. medicaginea*

Sample	TPC (mg GEA/g extract)	DPPH (IC_{50}, µg/ml)
Roots	19.62±0.55	479.21
Stems without chlorophyll removal	18.56±0.40	881.50
Leaves without chlorophyll removal	48.33±0.51	344.76
Stems with chlorophyll removal	17.52±0.75	1552.52
Leaves with chlorophyll removal	22.78±0.35	532.98

IC_{50} = 50% inhibitory concentration

Determination of antioxidant activity

Five different samples of methanolic extracts of *C. medicaginea* were investigated for antioxidant activity using DPPH free radical scavenging assay. All extracts exhibited high activity at the maximum concentration (2000 µg/ml) (Figure 1). Leaves extract without chlorophyll removal exhibited higher activity than other extracts, which showed a percentage of DPPH radical scavenging activity in the maximum concentration was 94.88±0.57%, followed by roots extract (91.23±0.74%). A percentage of DPPH radical scavenging activity of stems and leaves with chlorophyll removal was reduced from those without chlorophyll removal from 83.69±0.1 to 61.15±0.78% and 94.88±0.57 to 80.97±0.74%, respectively. It showed IC_{50} values in the extraction's DPPH radical scavenging activity assay (Table 2). The leaves without chlorophyll removal exhibited the highest antioxidant activity with an IC_{50} of 344.76 µg/ml.

Evaluation of antibacterial activity

The result showed roots extract exhibited more efficiency against five gram-positive bacteria, including *B. cereus, B. subtilis, K. rhizophila, S. aureus,* and *S. epidermidis,* as 10.03±0.03, 8.93±0.36, 9.61±0.66, 7.33±0.24 and

9.34±0.80 mm, respectively. Stems without chlorophyll removal showed antibacterial activity against *B. cereus*, *B. subtilis*, and *K. rhizophila* at 9.12±0.23, 7.10±0.15 and 7.88±0.3 mm, respectively. The extraction from leaves both with and without chlorophyll removal showed antibacterial activity against only *B. cereus* and *B. subtilis* as 7.39±0.22, 8.38±0.35 mm and 8.49±0.73, 9.95±0.39 mm, respectively. Nonetheless, all extracts were unable to against *E. coli* and *P. aeruginosa*.

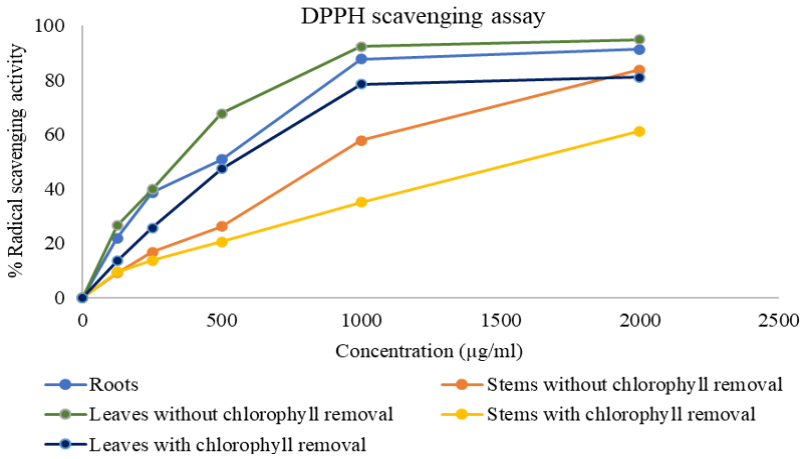

Figure 1. The percentage of DPPH radical scavenging activity from different parts of *C. medicaginea* at different concentrations

Phytochemical screening

The result showed that all extracts presented positive precipitation to Dragendorff's, Mayer's, Wagner's reagents and color changed to green-yellow or dark yellow when added 6M NaOH. The tannins test was examined using gelatin solution, gelatin salt solution, and 1% $FeCl_3$. The precipitation was shown with roots extract and slightly with leaves when added 1% $FeCl_3$. However, all extracts did not display saponin on the froth test (Table 3).

Table 3. Phytochemical screening for different methanolic extracts of *C. medicaginea*

Phytochemical	Test/ Reagent	Roots	Stems	Leaves
Alkaloid	Dragendorffs's	+	+	+
	Mayer's	+	+	+
	Wagner's	+	+	+
Tannin	Gelatin solution	+	-	-
	Gelatin salt solution	+	-	-
	1% FeCl3	+	-	+
Coumarin	6M NaOH	+	+	+
Saponin	Froth test	-	-	-

Where + = presences and - = absent

Discussion

Presently, the plant genus of *Crotalaria* is wildly used in traditional medicine. *C. medicaginea,* that local name is Phak Wan Ton, is one of the species which has never been reported for its biological activities of methanolic extract. Therefore, the study focused on the phytochemical activity, total phenolic content, antioxidant and antibacterial activities of different parts of *C. medicaginea*.

According to the results, the maceration method extracted three parts (roots, stems and leaves) of samples. Only stems and leaves were applied to remove chlorophyll using palm oil by liquid-liquid extraction because these presented a greenish-dark color which interfered with the resulting analysis (Namal Senanayake, 2013; Olatunde *et al*., 2018). The chlorophyll content in stems and leaves with chlorophyll removal of *C. medicaginea* was determined and compared to those of the extracts without chlorophyll removal. The stems and leaves with chlorophyll removal exhibited lower chlorophyll a, chlorophyll b and total chlorophyll content. Our results are related to a previous study that reported the dechlorophyllization of guava leaf extracts showed that the chlorophyll a, chlorophyll b and total chlorophyll content were reduced (Olatunde *et al*., 2018). Our results were consistent with those previously reported of *Piper betle* and *Piper sarmentosum* extracts with chlorophyll removal using a sedimentation method, exhibited a total chlorophyll content reduction by 23.08 and 10.19%, as documented by Tagrida and Benjakul (2020).

The total phenolic content in this study was determined using the Folin-Ciocalteu method. The leaves extract without chlorophyll removal showed higher total phenolic content of 48.33±0.51 mg GEA/g extract than other extracts. Our result was similar to the same species (*Crotalaria verrucosa* leaves extract), which exhibited a total phenolic content of approximately 41±

1.1 mg GEA/g extract (Sana *et al*., 2020). Likewise, the ethanolic extract of *Crotalaria spectabilis* exhibited total phenolic content of 40.8 ± 1.1 mg GEA/g extract (Suwanchaikasem *et al*., 2013). This study found that the stems and leaves extracted with chlorophyll removal presented lower than those without chlorophyll removal. According to Olatunde *et al*. (2018), the total phenolic content of ethanolic guava leaf extract with decholophyllization was decreased. Phaisan *et al*. (2020) described that the total phenolic content of *Chromolaena odorate* extract that removed chlorophyll from palm oils was lower than the control (without chlorophyll removal). It is similar to the decrease of total phenolic content in lead seed extracts treated by chlorophyll removal (Benjakul *et al*., 2014).

Furthermore, the result of the antioxidant activity is implicated in their total phenolic content. The leaves extract without chlorophyll removal showed the highest percentage of DPPH radical scavenging activity and exhibited the lowest IC_{50} in DPPH radical scavenging assay. Although the result of antioxidant activity from roots extract and leaves extract with chlorophyll removal showed slightly different total phenolic content. The free radical inhibition values remained nearby not only phenolic compounds that provided antioxidant activity but which non-phenolic antioxidants also donated to an activity (Banerjee *et al*., 2005). In previous studies, Devendra *et al*. (2012) reported the antioxidant activity of ethanolic leaf extracts from *Crotalaria* species. As a result, presented *C. medicaginea* showed IC_{50} at 74.0 µg/ml, which is lower activity than the extracts from this study. The low IC_{50} presented a more effective antioxidant, possibly the factors caused by the extraction solvents. In agreeing with Borges *et al*. (2020), who reported ethanol proved to be the best solvent to extract bioactive compounds with antioxidant properties of *Acacia dealbata* and *Olea europaea* compared with methanol.

On the other hand, the extracts may contain different phenolic compounds, which have different activities depending on the structure (Rahiman *et al*., 2012). The extracts with chlorophyll removal found that the percentage of DPPH radical scavenging activity was reduced in this finding. In contrast, Phaisan *et al*. (2020) reported using oils for the dechlorophyllization of *Chromolaena odorata*, indicating that palm oil and soybean oil gave high antioxidant activity compared with the control. However, our results were similar to the document of Tzima *et al*. (2020), they reported the antioxidant activity of rosemary and thyme with chlorophyll removal was examined with DPPH, ABTS and FRAP assays. They found the antioxidant activity of chlorophyll removal extracts was lower than the control in all assays.

In this study, the methanolic extract from the roots of *C. medicaginea* presented a higher potential for antibacterial activity with gram-positive

bacteria. In contrast, the extracts with chlorophyll removal exhibited lower antibacterial activity when compared to their extracts without chlorophyll removal. In a previous study, similar to our result, the ethanol and chloroform extracts of *C. medicaginea* exhibited high activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* at a concentration of 2.5 mg/disc (Devendra *et al*., 2012). In addition, Yadava and Vishwakarma (2014) reported the new allelochemicals from the methanolic extract from stems of *C. medicaginea* exhibited antibacterial activity against *E. coli* and *B. subtilis*. In comparison, our study presented that all extracts were unable to against gram-negative bacteria. Nevertheless, new allelochemical compounds had not inhibited *S. aureus*, which showed the same result in the stem extract of this study.

The phytochemical activity was investigated to screen the primary bioactive compounds in methanolic extracts from roots, stems and leaves without chlorophyll removal of *C. medicaginea.* This study showed all extracts presented alkaloid and coumarin compounds. The roots extract exhibited strong tannins. However, all extracts did not display saponins on the froth test. Besides, Devendra *et al*. (2012) found that both ethanol and chloroform extracts from leaves of *C. medicaginea* presented alkaloids and tannins. In addition, a previous study found alkaloids, tannins and coumarins in a methanolic extract with chlorophyll removal and without chlorophyll removal from the whole plant extracts of *C. medicaginea*. (Natsakulmong and Suwanmek, 2021). Therefore, our result is in accordance with both mentioned documents.

This study is the first report of biological activity from different parts of the methanolic extracts of *C. medicaginea*. The extract without chlorophyll removal had higher potential biological activity to total phenolic content, antioxidant and antibacterial activities compared to their extract with chlorophyll removal. However, antibacterial activity from the methanolic extract of *C. medicaginea* did not affect gram-negative bacteria. Therefore, *C. medicaginea* should be evaluated for biological activity with different solvents partition in further studies, which may help to develop food products, medicine and cosmetic applications.

Acknowledgments

This work was funded free from the School of Science and financially supported by King Mongkut's Institute of Technology Ladkrabang (grant number: A 118-0261-010). This project is a part of the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG). The samples were supported by Ban Wang Hora Community, Ong Phra Subdistrict, Dan Chang District, Suphanburi province.

References

Armania, N., Yazan, L. S., Musa, S. N., Ismail, I. S., Foo, J. B., Chan, C. H., Noreen, H., Hisyam, A. H., Zulfahmi, S. and Ismail, M. (2013). *Dillenia suffruticosa* exhibited antioxidant and cytotoxic activity through induction of apoptosis and G_2/M cell cycle arrest. Journal of Ethnopharmacology, 146:525-535.

Banerjee, A., Dasgupta, N. and De, B. (2005). In vitro study of antioxidant activity of *Syzygium cumini* fruit. Food Chemistry, 90:727-733.

Benjakul, S., Kittiphattanabawon, P., Sumpavapol, P. and Maqsood, S. (2014). Antioxidant activities of lead (*Leucaena leucocephala*) seed as affected by extraction solvent, prior dechlorophyllisation and drying methods. Journal of Food Science and Technology, 51:3026-3037.

Borges, A., José, H., Homem, V. and Simões, M. (2020). Comparison of techniques and solvents on the antimicrobial and antioxidant potential of extracts from *Acacia dealbata* and *Olea europaea*. Antibiotics, 9:48.

Breinholt, V., Bailey, G., Schimerlik, M. and Dashwood, R. (1995). Mechanisms of chlorophyllin anticarcinogenesis against Aflatoxin B1: Complex formation with the carcinogen. Chemical Research in Toxicology, 8:506-514.

Chantarasaka, S., Chareonsap, P. P. and Poeaim, S. (2022). Identification of phenolic compounds and evaluation of biological activities of methanolic extracts obtained from two varieties of longan (*Dimocarpus longan*) peels. International Journal of Agricultural Technology, 18:63-76.

Clinical and Laboratory Standards Institute. (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute, 32(2).

DeGraff, W. G. and Mitchell, J. B. (1987). Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity. Cancer Research, 47:943-946.

Denizot, F. and Lang, R. (1986). Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal of Immunological Methods, 89:271-277.

Devendra, B. N., Srinivas, N. and Solmon, K. S. (2012). A comparative pharmacological and phytochemical analysis of in vivo and in vitro propagated *Crotalaria* species. Asian Pacific Journal of Tropical Medicine, 5:37-41.

Elangovan, V., Sekar, N. and Govindasamy, S. (1994). Chemoprotective potential of dietary bipoflavonoids against 20-methylcholanthrene-induced tumorigenesis. Cancer Letters, 87:107-113.

Ferruzzi, M. G. and Blakeslee, J. (2007). Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. Epidemiologic Nutrition Research, 27:1-12.

Hörtensteiner, S. and Kräutler, B. (2011). Chlorophyll breakdown in higher plants. Biochimica et Biophysica Acta – Bioenergetics, 1807:977-988.

Jain, S. C., Jain, R. and Singh, R. (2009). Ethnobotanical survey of Sariska and Siliserh regions from Alwar district of Rajasthan, India. Ethnobotanical Leaflets, 13:171-188.

Kwartiningsih, E., Ramadhani, A. N., Putri, N. G. A., and Damara, V. C. J. (2021). Chlorophyll extraction methods review and chlorophyll stability of Katuk Leaves (*Sauropus androgynous*). Journal of Physics: Conference Series, 1858:12-15.

Lordkhem, P. (2016). Bioactivity and genetic diversity of *Phlogacanyhus pulcherrimus*. (Master Thesis). King Mongkut's Institute of Technology Ladkrabang, Thailand.

Namal Senanayake, S. P. J. (2013). Green tea extract: chemistry, antioxidant properties and food applications - A review. Journal of Functional Foods, 5:1529-1541.

Natsakulmong, P. and Suwanmek, W. (2021). Species identification of *Crotalaria* sp. and evaluation of phytochemical screening. (Bachelor Thesis). King Mongkut's Institute of Technology Ladkrabang, Thailand.

Ngoci, S. N., Mwendia, C. M. and Mwaniki, C. G. (2011). Phytochemical and cytotoxicity testing of *Indigofera lupatana* Baker F. Journal of Animal and Plant Sciences, 11:1364-1373.

Ninkaew, S., Balslev, H. and Chantaranothai, P. (2018). *Crotalaria* L. In: K. Chayamarit et al. (eds), Flora of Thailand. The Forest Herbarium, Bangkok, 4:260-261.

Niyomdham, C. (1978). A revision of the genus *Crotalaria* Linn. (Papilionoideae) plants. Thai Forest Bulletin (Botany), 11:105-181.

Olatunde, O. O., Benjakul, S. and Vongkamjan, K. (2018). Antioxidant and antibacterial properties of guava leaf extracts as affected by solvents used for prior dechlorophyllization. Journal of Food Biochemistry, 42:1-11.

Phaisan, S., Yusakul, G., Sakdamas, A., Taluengjit, N., Sakamoto, S. and Putalun, W. (2020). A green and effective method using oils to remove chlorophyll from *Chromolaena odorata* (L.) R.M. King and H. Rob. Songklanakarin Journal of Science and Technology, 42:1084-1090.

Rahiman, S., Tantry, B. A. and Kumar, A. (2012). Variation of antioxidant activity and phenolic content of some common home remedies with storage time. African Journal of Traditional, Complementary, and Alternative Medicines: AJTCAM / African Networks on Ethnomedicines, 10:124-127.

Sana, S. S., Kumbhakar, D. V., Pasha, A., Pawar, S. C., Grace, A. N., Singh, R. P., Nguyen, V. H., Le, Q. van. and Peng, W. (2020). *Crotalaria verrucosa* leaf extract mediated synthesis of Zinc Oxide nanoparticles: assessment of antimicrobial and anticancer activity. Molecules (Basel, Switzerland), 25:1-21.

Sreeprasert, J. (2016). Phytochemical screening and biological activity of *Dolichandrone Serrulata*. (Master Thesis). University of Burapha, Thailand.

Stavric, B. (1994). Antimutagens and anticarcinogens in foods. Food and Chemical Toxicology, 32:79-90.

Suwanchaikasem, P., Phadungcharoen, T. and Sukrong, S. (2013). Authentication of the Thai medicinal plants sharing the same common name "Rang Chuet": Thunbergia laurifolia, *Crotalaria spectabilis*, and *Curcuma* aff. *amada* by combined techniques of TLC, PCR-RFLP fingerprints, and antioxidant activities. Science Asia, 39:124-133.

Tagrida, M. and Benjakul, S. (2020). Ethanolic extract of Betel (*Piper betle* L.) and Chaphlu (*Piper sarmentosum* Roxb.) dechlorophyllized using sedimentation process: production, characteristics, and antioxidant activities. Journal of Food Biochemistry, 44:1-14.

Thiankathet, C., Luengsawet, P. and Bututham, A. (2019). Preliminary on the bioactivity of methanolic extract from santol. (Bachelor degree). King Mongkut's Institute of Technology Ladkrabang, Thailand.

Tzima, K., Brunton, N. P. and Rai, D. K. (2020). Evaluation of the impact of chlorophyll removal techniques on polyphenols in rosemary and thyme by-products. Journal of Food Biochemistry, 44:1-22.

Yadava, R. N. and Vishwakarma, U. K. (2014). New potential allelochemicals from *Crotalaria medicaginea* Lam. Chemical Science Transactions, 3:141-150.

Yin, M. C. and Cheng, W. S. (1998). Inhibition of *Aspergillus niger* and *Aspergillus flavus* by some herbs and spices. Journal of Food Protection, 61:123-125.

(Received: 21 October 2022, accepted: 30 December 2022)