Effect of extraction methods on yield, total phenolic content and antioxidant activity of *Ipomoea pes-caprea* (L.) R. Br. leaves

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Abstract

Ipomoea pes-caprea (L.) R. Br. is traditionally used in nutrition, medicine, and agriculture in Thailand to treat inflammation of toxic effects from the jellyfish venoms and dermatitis. However, I. *pes-caprae* still has a limited study on the selection of suitable extraction techniques for upscaling purposes. This study aims to use different extraction methods: maceration assisted extraction, ultrasound-assisted extraction, microwave-assisted extraction, and espresso machine for evaluating the yield, total phenolic contents as well as determining antioxidant activity (DPPH and ABTS scavenging) from I. *pes-caprae* leaves. Each part of the plant was extracted by reflux using *five* different methods such as maceration, microwave, ultrasound, and espresso machine. Total phenolic content was determined with Folin-Ciocalteu 1:10 on 765 nm using a microplate reader. Antioxidant activity was determined using DPPH and ABTS free radical scavenger methods. The yield of I. *pes-caprae* extracts isolated with different methods showed that the highest yield was associated with 50% EtOH maceration extraction (18.60%). Total phenols content and DPPH free radical scavenging activity were the highest value in 50% EtOH with microwave 5 min (64.26±0.33 mgGAE/g and IC_{50} 24.07±0.03 µg/mL respectively). The crude extracts displayed ABTS free radical scavenging activity with the highest value in 25% EtOH espresso machine (IC_{50} 488.71±0.42 µg/mL). This study demonstrates that microwave-assisted extraction for 5 minutes with 50% EtOH of *pes-caprae* leaves showed the most compromising result in terms of the efficient extraction and recommended for a potential source of natural antioxidants and high total phenolic content for commercial uses.

***Keywords*:** *Ipomoea pes-caprea* (L.) R. Br., *Industrial scale, Total phenolic content, DPPH, ABTS*

1. Introduction

Jellyfish is becoming a major public health concern in Thailand. Thailand has reported that poisonous jellyfish appear and increase the number of outbreaks continuously. Noticed from seeing the warning signs when going to the sea to watch out for jellyfish. In 2018, the test for vinegar and seawater on a live bluebottle was reported to inhibit the release of jellyfish nematocysts. A local plant in Thailand, *Ipomoea pes-caprae* or morning glory, has successfully treated jellyfish poisoning stings. (Sucharitakul et al., 2018; Premmaneesakul & Sithisarankul, 2019).

Ipomoea pes-caprae (L.) R.br. is a traditional use in nutrition, medicine, and agriculture. In Thailand, folk wisdom uses fresh leaves to treat inflammation of toxic effects from the jellyfish venoms, dermatitis and has scientific evidence found in the mechanisms of reduction of prostaglandin and leukotriene formation (De Souza et al., 2000; Meira et al., 2012). The important substance in *Ipomoea pes-caprae* (L.) R.br. such as β-damascenone and E-phytol have equivalent to a drug called papaverine. Also, antihistamine activity studies were tested with the intestines stimulated by histamine and jellyfish poisoning and found that β-damascenone and E-phytol effectively inhibited. (Pongprayoon et al., 1992 and Phoenix et al., 1989). Bioactive ingredients such as quercetin 3-O-β-D-glucofuranoside, β-amyrin acetate, α-amyrin acetate, betulinic acid, glochidone, and platelet (14C) 5-hydroxytryptamine (5-HT) of *Ipomoea pes-caprae* (L.) R.br. have efficacy in migraine attack therapy, anti-hemolytic, anti-inflammatory, and antihistamine activities in mice. (Pongprayoon et al., 1992). Moreover, crude extracts of *Ipomoea pes-caprae* (L.) R.br. leaves from petroleum ether extract with a water-steam distillate can inhibit prostaglandin synthesis in vitro and has efficiency treatment inflammation of toxic effect from the jellyfish venoms in the preliminary clinical study (Pongprayoon et al., 1991). Moreover, researchers have processed two *Ipomoea pes-caprae* (L.) R.br.

extracted products such as balm IPA to relieve inflammation, pain, swelling from insect bites, and 1% extracted cream to decrease tissue destruction caused by jellyfish poisoning (Sunthonpalin & Wasuwat, 1985).

In Thailand, *Ipomoea pes-caprae* plants or "beach morning glory" are abundant but the products that contain *Ipomoea pes-caprae* (L.) R. Br. leaf extracts imported from foreign countries for treating allergies and scars, which including production or standardization within Thailand, are not well established. High yield extraction and quality control are very important to fill up Thai Pharmacopoeia and further enhance the development of the product to treat jellyfish venoms in the future. At the manufacturing enterprise-level, significant advance technology has been made in the transforming of medicinal plants such as the concurrent extraction methods, ultrasound-assisted extraction, Supercritical fluid extraction and, microwave-assisted in which these advances are schemed to increase yield, green environment with high efficacy at a lower cost. With such a variety of methods present in traditional and modern use, the selection of capable extraction methods needs scrupulous evaluation. Therefore, our research is interested in studying the comparison of different extraction methods in *Ipomoea pes-caprae* (L.) R. Br. leaves for industrial scale.

2. Objectives

This study aims to extract *Ipomoea pes-caprae* (L.) R.br. with different methods for applying in the industrial scale and determine the phenolic content and antioxidant activity of *Ipomoea pes-caprae* (L.) R.br.

3. Materials and Methods

Plant materials

The leaves of I. *pes-caprae* were collected from Chonburi, Thailand (Figure 1). Clean the leaves with water, air dry, and a hot air oven was used to dry at 60 ºC for 24 hr. After drying, a blender mill was used to ground each set of each plant material, and sieving was used to determine the average size. (Pongprayoon et al., 1992).

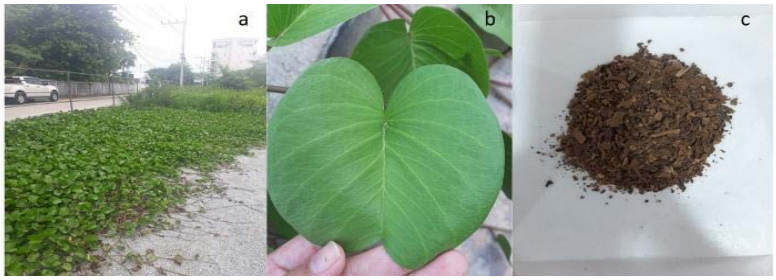

Figure 1 (a) *Ipomoea pes-caprea* (L.) R. Br. leaves from Chonburi, Thailand; (b) *Ipomoea pes-caprea* (L.) R. Br. leaves; (c) leaves powder

Extraction of Bioactive compounds with different methods

Scientists have studied and analyzed the impact of different types of solvents on the extraction of highly active ingredient yields and effectively at extracting antioxidants such as phenolics, flavonoids, alkaloids, and terpenoids (Ammar et al., 2017 and Truong et al., 2019). Most previous studies have discovered that highly polar solvents, such as ethanol and methanol, have high effectiveness to separate antioxidant compounds (Ammar et al., 2017). This study was performed to study the high quantity of extraction methods and determine the phenolic content and antioxidant activity of *Ipomoea pes-caprae* (L.) R.br. Therefore, the I. *pes-caprae* leaves solvent for extraction were prepared according to the protocol of Umamaheshwari et al. (2012) and Daniela et al. (2012) with some modifications by using ethanol at different ratios with water.

1) Maceration assisted extraction (Yeo et al., 2014)

Soaked 20 g leaves and 200 mL solvent with 50% ethanol and 100% ethanol mixtures, then a Whatman No. 1 filter paper and a rotary evaporator were used to filter and evaporate at 60 ºC, respectively.

2) Microwave-assisted extraction (Rincón et al., 2019)

50% EtOH and 100% EtOH (200 mL) were used as a solvent to extract 20 g leaves with a combination of seven extraction times (15, 30, 35, 40, 45, 60 and 75 minutes) performed at 100W microwave. After the process, extracts were filtered through a filter paper and increased the concentration with a rotary evaporator at 60 ºC.

3) Ultrasound-assisted extraction (Rincón et al., 2019)

2 g extraction of dried leaves were separated by 80 mL of 50% EtOH and 100% EtOH solvent by an ultrasonic bath system of 360W and 50/60 kHz. Multiple extractions at various extracting times (60, 120, and 150 minutes) have been made to determine the proper time of extraction. Extracts were collected, then filtered, evaporated with a rotary evaporator at 60 ºC.

4) Espresso Machine (Just et al., 2016)

The portafilter of the Espresso machine was packed with 3 g leaves of I. *pes-caprae* using a coffee tamper. The leaves were extracted with the solvent (~100 mL) and collected in a beaker for 20 times. Filtered through a filter paper, and a rotary evaporator has been used at 60 ºC.

Evaluation of antioxidant activity

(1) Determination of total phenolic content (Sembiring et al., 2018)

A total of 20 µL of the diluted extract of the sample was mixed with 100 µL of 1:10 diluted Folin–Ciocalteu reagent and added with 80 µL of sodium carbonate solution (100 g/L) into the plate. The solution was then Incubated for 30 minutes in the dark and using a microplate reader measurement absorbance at 750 nm. Gallic acid was used for the standard dilutions (from 3.12, 6.25, 12.5, 25, 50, and 100 µg/mL). Total phenolic contents were expressed as mg Gallic Acid Equivalents (GAE) per g of plant extract.

(2) 2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH assay) (Boly et al., 2016)

100 µL of different concentrations of extract in methanol (from 1.5625, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL) were added to 100 µL of the methanol DPPH solution. The solutions were then placed in the incubated plate for 30 minutes in the dark and measured using a microplate reader at 540 nm. Gallic acid was also used for the standard dilutions (from 1.5625, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL). The DPPH radical scavenging activity (%) was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(A - (B - C)) / A] \times 100$$

where A—absorption of blank sample [DPPH + methanol without sample], B—absorption of tested extract solution [DPPH + sample (extract/standard)], and C—absorption of sample blank [sample (extract/standard) + methanol without DPPH]

(3) 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay (ABTS assay) (Lee et al., 2015)

7 mM ABTS were mixed with 2.45 mM Potassium persulfate and kept in the dark at room temperature for 12-16 hours to allow free radical generation and were then diluted with methanol (1 : 15, v/v). After that, 100 µL ABTS reagent were mixd with 50 µL of the sample in a 96-well microplate. The solution was incubated at room temperature for 15 minutes, then measured its absorbanceusing a microplate reader at 734 nm.

$$\text{\% ABTS inhibition} = [(A - (B - C)) / A] \times 100$$

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where, A—absorption of blank sample [ABTS + methanol without sample], B—absorption of tested extract solution [ABTS + sample (extract/standard)], and C—absorption of sample blank [sample (extract/standard) + methanol without ABTS]

4. Results and Discussion

Percentage Yield of Extract

The results showed that 50% EtOH maceration extraction (18.60%) was the highest yield, followed by 50% EtOH microwave 5 minutes (15.33%), 25% EtOH espresso machine (12.67%), 50% EtOH ultrasonic 120 minutes (5.50%), 100% EtOH ultrasonic 150 minutes (1.25%), 100% EtOH microwave 60 minutes (0.67%), and then 100% EtOH maceration extraction (0.80%) (Table1).

The desired extractive technique is the most important to obtain effective bioactive compounds with high yield extraction and lower cost. Bioactive compounds are dependent on different method techniques. Therefore, it is necessary to select a suitable extraction method to improve the manufacturing of advanced innovative products in the future (Quispe- Candori et al., 2008).

Although the maceration technique had the highest efficiency, this process requires two weeks for extraction. In contrast, the samples obtained with microwave extraction time could be extracted in 5 minutes. The previous study of some medicinal herbs treated with the microwave (Rincón et al., 2019) and maceration extraction (Yeo et al., 2014) achieved higher extraction yield than using steam distillation (Pongprayoon et al., 1992).

Evaluation of extraction techniques for I. *pes-caprae* found extraction yields in the order of maceration > microwave > steam distillation, where the higher recovery in the microwave samples can be associated with temperature, types of solvents during the extraction, and duration of extraction. This study will show that the highest yield was associated with 50% EtOH maceration extraction.

Table 1 Yield of I. *pes-caprae* extracts with different methods

Method	**% yield (w/w)**		
	100% EtOH	50% EtOH	25% EtOH
Maceration extraction	0.80	18.60	-
Microwave 5 min	-	15.33	-
Microwave 60 min	0.67	-	-
Ultrasonic 120 min	-	5.50	-
Ultrasonic 150 min	1.25	-	-
Espresso Machine	-	-	12.67

Total Phenolic Content

The gallic acid calibration curve has shown at 765 nm wavelength (equation $y = 5.0871x + 0.0399$, $R^2 = 0.9977$).

The total phenol contents of eight crude extracts, 50% EtOH microwave 5 minutes contained the highest (64.26±0.33 mgGAE/g) amount of total phenolic content compounds followed by 50% EtOH ultrasonic 120 minutes (63.40±0.93 mgGAE/g), 100% EtOH microwave 60 minutes (51.00±0.07 mgGAE/g), 25% EtOH espresso machine (32.72±0.11 mgGAE/g), 50% EtOH maceration extraction (30.71±0.04 mgGAE/g), 100% EtOH ultrasonic 150 minutes (25.92±0.08 mgGAE/g), and then 100% EtOH maceration extraction (24.55±0.72 mgGAE/g) (Table2).

Table 2 Total phenolic content of I. *pes-caprae* extracts isolated with different methods

Method	**Total Phenolic Content (mgGAE/gram)**			
	STD	100% EtOH	50% EtOH	25% EtOH
Gallic acid	9.82±0.01			
Maceration extraction	-	24.55±0.72	30.71±0.04	-
Microwave 5 min	-	-	64.26±0.33	-
Microwave 60 min	-	51.00±0.07	-	-

Method	Total Phenolic Content (mgGAE/gram)			
	STD	100% EtOH	50% EtOH	25% EtOH
Ultrasonic 120 min	-	-	63.40±0.93	-
Ultrasonic 150 min	-	25.92±0.08	-	-
Espresso Machine	-	-	-	32.72±0.11

A Mean ± SD, n = 3

DPPH radical scavenging

The 50% EtOH microwave 5 minutes (IC_{50} 9.05±0.13 µg/mL) has the highest ability to scavenge free radicals followed by 50% EtOH maceration extraction (IC_{50} 24.07±0.03 µg/mL), 50% EtOH ultrasonic 120 minutes (IC_{50} 30.33±0.25 µg/mL), 100% EtOH microwave 60 minutes (IC_{50} 48.00±0.65 µg/mL), 25% EtOH espresso machine (IC_{50} 117.31±0.16 µg/mL), 100% EtOH ultrasonic 150 minutes (IC_{50} 118.94±0.70 µg/mL), and then 100% EtOH maceration extraction (IC_{50} 154.46±0.00 µg/mL) (Table 3).

Table 3 Antioxidant DPPH scavenging activity of I. *pes-caprae* extracts isolated with a different method

Method	IC_{50} (µg/mL)			
	STD	100% EtOH	50% EtOH	25% EtOH
Gallic acid	18.94±0.26			
Maceration extraction	-	154.46±0.00	24.07±0.03	-
Microwave 5 min	-	-	9.05±0.13	-
Microwave 60 min	-	48.00±0.65	-	-
Ultrasonic 120 min	-	-	30.33±0.25	-
Ultrasonic 150 min	-	118.94±0.70	-	-
Espresso Machine	-	-	-	117.31±0.16

A Mean ± SD, n = 3

ABTS radical scavenging

The 25% EtOH espresso machine (IC_{50} 488.71±0.42 µg/mL), has shown the highest ability to scavenge free radicals, followed by 50% EtOH ultrasonic 120 minutes (IC_{50} 506.34±0.44 µg/mL), 50% EtOH microwave 5 minutes (IC_{50} 508.81±0.33 µg/mL), 50% EtOH maceration extraction (IC_{50} 539.27±0.07 µg/mL), 100% EtOH ultrasonic 150 minutes (IC_{50} 1109.15±0.36 µg/mL), and then 100% EtOH microwave 60 minutes (IC_{50} 1141.74±0.08 µg/mL) (Table 4).

Table 4 ABTS absorption inhibition (%) of I. *pes-caprae* extracts isolated with a different method

Method	IC_{50} (µg/mL)			
	STD	100% EtOH	50% EtOH	25% EtOH
Ascorbic acid	24.44±0.02			
Maceration extraction	-	-	539.27±0.07	-
Microwave 5 min	-	-	508.81±0.33	-
Microwave 60 min	-	1141.74±0.08	-	-
Ultrasonic 120 min	-	-	506.34±0.44	-
Ultrasonic 150 min	-	1109.15±0.36	-	-
Espresso Machine	-	-	-	488.71±0.42

A Mean ± SD, n = 3

In this study, the effects of different extraction techniques altered the order of decreasing antioxidant activity among the I. *pes-caprae* extracts as follows 50% EtOH microwave > 50% EtOH maceration extraction > 50% EtOH ultrasonic > 100% EtOH microwave > 25% EtOH espresso machine > 100% EtOH ultrasonic > 100% EtOH maceration. This result indicates that 50% EtOH solvent with a microwave approach is the optimum procedure for separating the highest antioxidant compounds as well as the highest amount of total phenolic compounds from I. *pes-caprae* leaves. Phenolic acids and flavonoids are ubiquitous bioactive compounds that belong to a diverse group of secondary metabolites and are universally present in higher

plants. These phytochemicals have shown to possess significant antioxidant capacities that can protect the human body from health problems (Robards et al., 1999). Polyphenols are often most soluble in organic solvents less polar than water. The solubility is dependent on the polar properties of the polyphenols (Kim and Lee , 2001). Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the active molecules of interest. A solvent of similar polarity to the active compounds will properly dissolve (Ammar et al., 2017 and Truong et al., 2019). From the varying ratio of ethanol: water at 100%, 50% and 25%, the bioactive compounds of I. *pes-caprae* leaves are favorable solubility at the moderately polar solvent of 50% ethanol.

Microwave extraction is used as an alternative to traditional extraction methods such as soxhlet, percolation, digestion, and maceration technique, etc. Because microwave extraction has affected power on the extraction efficacy. Microwave power approved the solvent's movement into the cell plant, increasing the extraction efficacy, less extraction time, and low temperature (Li et al., 2017). The traditional method, maceration technique is commonly used by intending to soften and break the plant's cell wall to release the soluble high amount of phytochemicals but this process has a long duration time and a large volume of organic waste (Yeo et al., 2014). While ultrasound extraction is used to disrupt plant cell walls, which helps improve the solvent's ability to penetrate the cells and obtain a higher extraction yield (Ammar et al., 2017 and Rincón et al., 2019), however, use of high ultrasound energy may have an effect on the active phytochemicals through the formation of free radicals (Azwanida, 2015). Lastly, espresso machines used the high pressurized and high temperature solvent extraction system with a short time to separate the active ingredients from plants (Just et al., 2016 and Safdar et al., 2017). In principle, ultrasound extraction and espresso systems should have high recoveries for our study but the yield of the extract depends on parameters like solvent concentration, extraction temperature, time and type of plant. Therefore microwave and maceration extraction achieved a higher extraction yield than other methods for extract I. pes-caprae leaves.

We compare the total phenolic content of the eight crude extracts in this study as a similar aqueous extract of the previous study. Antioxidant radical scavenging activity of the eight crude extracts in this study was higher than the antioxidant status of the chloroform and ethyl acetate extracts (Banerjee et al., 2013). Seven methods of I. *pes-caprae* were able to decolorize DPPH, ABTS and the free radical scavenging potentials of the extracts were found damascenone, E-phytol, eugenol, quercetin 3-O-β-D-glucofuranoside, β-amyrin acetate, α-amyrin acetate, betulinic acid, glochidone, and platelet (14C) 5-hydroxytryptamine (5-HT) (Pongprayoon *et al.,* 1992, Phoenix *et al.,* 1989) All these compounds isolated from natural sources were shown to possess anti-inflammatory activity. I. *pes-caprae* with high antioxidant activity might be proposed for impeding toxic effect from the jellyfish venoms, dermatitis and has scientific evidence found the mechanisms of reduction of prostaglandin and leukotriene formation (De Souza et al., 2000; Meira et al., 2012).

Antioxidant activity of the extracts analyzed and determined by radical scavenging activities and reducing power. DPPH is one of the most widely reported techniques for measuring antioxidant activity. The DPPH assay determines a change in antioxidants that could be polar, such as phenolics and flavonoids (Rahman et al., 2015).

The ABTS assay uses ABTS radicals performed by oxidation of ABTS with potassium persulphate. Antioxidant compounds and ABTS react to form a radical cation, ABTS+ . In this study, DPPH has strong antioxidant activity better than ABTS (Tai et al., 2016). The crude extract may be rich in the total phenolic content of the extractives with free radical scavenging efficiencies.

5. Conclusion

In this study, 50% EtOH maceration had the highest yield, 50% EtOH microwave 5 min had the highest amount of total phenolic content and ability to scavenge free radicals, and 25% EtOH espresso machine had the highest ability to scavenge free radicals. The assessment of antioxidant activity indicated that I. *pes-caprae* with higher phenolic and antioxidant activity could be a significant source of natural antioxidants. The quantification of antioxidant properties could serve as a guide for the use of these plants to treat inflammation. Further investigation into the isolation and identification of responsible antioxidant

components with HPLC and their mechanism of action is necessary to understand better their ability to the reduction of prostaglandin that has a significant impact on the inflammation machinery.

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7. References

Ammar, A., Naoufal, L., Azam, B., Dennis, G.W., & David A.L. (2017). Phytochemicals: Extraction, Isolation,and Identification of Bioactive Compounds from Plant Extracts. *Plants*, 6, 1-23.

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength, and limitation. *Med Aromat Plants*, *4*(196), 2167-0412.

Banerjee, D., Hazra, A. K., Seal, T., Sur, T. K., Bhattacharyya, D., & Ray, J. (2013). Antioxidant and anti-inflammatory activities of different solvent extracts and isolated compounds of *Ipomoea pes-caprae* (L) sweet of Sunderban mangrove eco-complex. *Asian J Chem*, *25*, 4997-5000.

Boly, R., Lamkami, T., Lompo, M., Dubois, J., & Guissou, I. (2016). DPPH free radical scavenging activity of two extracts from *Agelanthus dodoneifolius (Loranthaceae)* leaves. *International Journal of Toxicological and Pharmacological Research*, 8(1), 29-34.

De Souza, M. M., Madeira, A., Berti, C., Krogh, R., Yunes, R. A., & Cechinel-Filho, V. (2000). Antinociceptive properties of the methanolic extract obtained from *Ipomoea pes-caprae* (L.) R. Br. *Journal of Ethnopharmacology*, *69*(1), 85-90.

Just, J., Bunton, G. L., Deans, B. J., Murray, N. L., Bissember, A. C., & Smith, J. A. (2016). Extraction of eugenol from cloves using an unmodified household espresso machine: an alternative to traditional steam-distillation. *Journal of Chemical Education*, 93(1), 213-216.

Kim, D. O., Lee, C. Y (2001) Extraction and isolation of polyphenolics. Current Protocols in Food Analytical Chemistry: John Wiley & Sons, Inc

Lee, K. J., Oh, Y. C., Cho, W. K., & Ma, J. Y. (2015). Antioxidant and anti-inflammatory activity determination of one hundred kinds of pure chemical compounds using offline and online screening HPLC assay. Evidence-Based Complementary and Alternative Medicine, 2015.

Li, Y., Li, S., Lin, S. J., Zhang, J. J., Zhao, C. N., & Li, H. B. (2017). Microwave-assisted extraction of natural antioxidants from the exotic Gordonia axillaris fruit: Optimization and identification of phenolic compounds. *Molecules*, *22*(9), 1481.

Meira, M., Silva, E. P. D., David, J. M., & David, J. P. (2012). Review of the genus *Ipomoea*: traditional uses, chemistry and biological activities. *Revista Brasileira de Farmacognosia*, *22*(3), 682-713.

Phoenix, J., Edwards, R. H., & Jackson, M. J. (1989). Inhibition of Ca^{2+}-induced cytosolic enzyme efflux from skeletal muscle by vitamin E and related compounds. *Biochemical Journal*, *257*(1), 207-213.

Pongprayoon, U., Baeckström, P., Jacobsson, U., Lindström, M., & Bohlin, L. (1991). Compounds inhibiting prostaglandin synthesis isolated from *Ipomoea pes-caprae*. *Planta medica*, *57*(06), 515-518.

Pongprayoon, U., Baeckström, P., Jacobsson, U., Lindström, M., & Bohlin, L. (1992). Antispasmodic activity of β-damascenone and E-phytol isolated from *Ipomoea pes-caprae*. *Planta Medica*, *58*(01), 19-21.

Premmaneesakul, H., & Sithisarankul, P. (2019). Toxic jellyfish in Thailand. *International Maritime Health*, *70*(1), 22-26.

Quispe-Condori, S., Foglio, M. A., Rosa, P. T., & Meireles, M. A. A. (2008). Obtaining β-caryophyllene from Cordia verbenacea de Candolle by supercritical fluid extraction. *The Journal of Supercritical Fluids*, *46*(1), 27-32.

Rahman, M. M., Islam, M. B., Biswas, M., & Alam, A. K. (2015). In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. *BMC research notes*, *8*(1), 621.

Rincón, E., Balu, A. M., Luque, R., & Serrano, L. (2019). Mechanochemical extraction of antioxidant phenolic compounds from Mediterranean and medicinal Laurus nobilis: A comparative study with other traditional and green novel techniques. Industrial Crops and Products, 141, 111805.

Robards, K., Prenzler, PD., Tucker, G., Swatsitang, P., Glover, W.,(1999) Phenolic compounds and their role in oxidative processes in fruits. Food Chem. ;66:401–436.

Safdar, M. N., Kausar, T., & Nadeem, M. (2017). Comparison of ultrasound and maceration techniques for the extraction of polyphenols from the mango peel. *Journal of Food Processing and Preservation*, *41*(4), e13028.

Sembiring, E. N., Elya, B., & Sauriasari, R. (2018). Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of Caesalpinia bonduc (L.) Roxb. *Pharmacognosy Journal*, 10(1)

Sucharitakul, P., Chomdej, S., Achalawitkun, T., Aongsara, S., Arsiranant, I., Paiphongpheaw, P., & Chanachon, K. (2018). Chirodropid box jellyfish in the Gulf of Thailand. *Marine Biodiversity*, 1-6.

Sunthonpalin, P. & Wasuwat, S. (1985). Jellyfish dermatitis treated by the extract of *Ipomoea pes-caprae*. *Siriraj Hospital Gazette* 37, 329-338.

Tai, A., Ohno, A., & Ito, H. (2016). Isolation and characterization of the 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) radical cation-scavenging reaction products of arbutin. *Journal of agricultural and food chemistry*, *64*(38), 7285-7290.

Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., & Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of Severinia buxifolia. *Journal of food quality*, *2019*.

Yeo, Y. L., Chia, Y. Y., Lee, C. H., Sow, H. S., & Yap, W. S. (2014). Effectiveness of maceration periods with different extraction solvents on in-vitro antimicrobial activity from fruit of *Momordica charantia* L. Journal of Applied Pharmaceutical Science, 4(10), 16-23.