ผลของตัวทำละลายต่อองค์ประกอบทางพฤกษเคมีของเสม็ดขาวและเสม็ดแดง

Solvent Effect on Phytochemical Screening of *Melaleuca leucadendra* Linn. and *Syzygium cinerea*

สุนันทา ข้องสาย * และ ลักษมี วิทยา
Sunanta Khongsai * and Luksamee Vittaya
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บทคัดย่อ

การศึกษาองค์ประกอบทางพฤกษเคมีเป็นขั้นตอนสำคัญที่นำไปสู่การแยกสารสำคัญและพบสารใหม่ งานวิจัยนี้ มีวัตถุประสงค์เพื่อศึกษาองค์ประกอบทางพฤกษเคมีเบื้องต้นของสารสกัดจากใบ ดอก และผลของเสม็ดขาว และเสม็ดแดงซึ่งเป็นพืชยืนต้นที่อยู่ในวงศ์ MYRTACEAE เตรียมสารสกัดโดยการสกัดในตัวทำละลายตามลำดับความเป็นขั้ว ได้แก่ เฮกเซน เอทธิลอะซิเตต เอทานอล และเมทานอล ตามลำดับ พบสารสำคัญในกลุ่มแอนทราควิโนน เทอร์พีนอยด์ ฟลาโวนอยด์ ซาโปนิน แทนนิน และแอลคาลอยด์ในสารสกัดหยาบจากดอกและผลของเสม็ดขาว ที่สกัดด้วยตัวทำละลายเอทิลอะซิเตต เอทานอล และเมทานอล และสารสกัดหยาบจากใบเสม็ดขาว ใบ และดอกของเสม็ดแดงที่สกัดด้วยตัวทำละลายเอทานอลและเมทานอล

คำสำคัญ: องค์ประกอบทางพฤกษเคมี, เสม็ดขาว, เสม็ดแดง

ABSTRACT

Phytochemical screening is an important procedure forthe isolation of new compounds. This research aimed to screen phytochemicals in leaves, flowers and fruits of *Melaleuca leucadendra* and *Syzygium cinerea* which belong to the family Myrtaceae. Extracts of the plant material were prepared using various solvents in the order of their polarity, including hexane, ethyl acetate, ethanol and methanol, respectively. Phytochemical screening of the extracts revealed the presence of anthraquinones, terpenoids, flavonoids, saponins, tannins and alkaloids in ethyl acetate, ethanol and methanol extracts of flowers and fruits of *M. Leucadendra*, including in ethanol and methanol extracts of leaves of this plant as well as in leaves and flowers of *S. cinerea*.

Key words: phytochemical constituents, *Melaleuca leucadendra*, *Syzygium cinerea*

คณะวิทยาศาสตร์และเทคโนโลยีการประมง มหาวิทยาลัยเทคโนโลยีราชมงคลศรีวิชัย อำเภอสิเกา จังหวัดตรัง 92150

Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Sikao, Trang 92150, Thailand.

* Corresponding author, e-mail: Ta047@hotmail.com

INTRODUCTION

Plants are now important target in allopathic medicine, herbal medicine, homoeopathy and aromatherapy. They have special function comprising to different groups, such as antioxidant, antispasmodics, anticancer and antimicrobials etc. The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites, such as alkaloids, steroids, flavonoids, tannins and phenolic compoundswhich are capable of producing definite physiological action on body (Raheela *et al*., 2012).

Phytochemistry deals with the chemistry of plant metabolites and their derivatives. Most of plant compounds that have found to be medicinally useful and interesting tend to be secondary metabolites (Ameenah, 2006). Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids prevent against chronic diseases. Saponins inhibit hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic properties. The steroids and saponins are responsible for central nervous system activities (Prashant *et al*., 2011). Phytochemical studies are of interest to plant scientists due to new and sophisticated drug discoveries (Ovuakporie and MacDonald, 2016). A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the protective effect of herbal plants has been attributed by phytochemicals, which are the non-nutrient compounds.

The composition of the metabolites are various according to the area of growth, the soil, the weather conditions, the time of harvest, the processing, the part of the plant, the time of extraction, and the solvents used in that extraction will all have a significant implication on the final chemical composition (Anthony *et al*., 2009).

In this study, leaves, flowers and fruits of two traditionally medicinal plants, *Melaleuca leucadendra* and *Syzygium cinerea* belonging to the family Myrtaceae were selected to investigate the solvent effect on phytochemical constituents.

MATERIALS AND METHODS

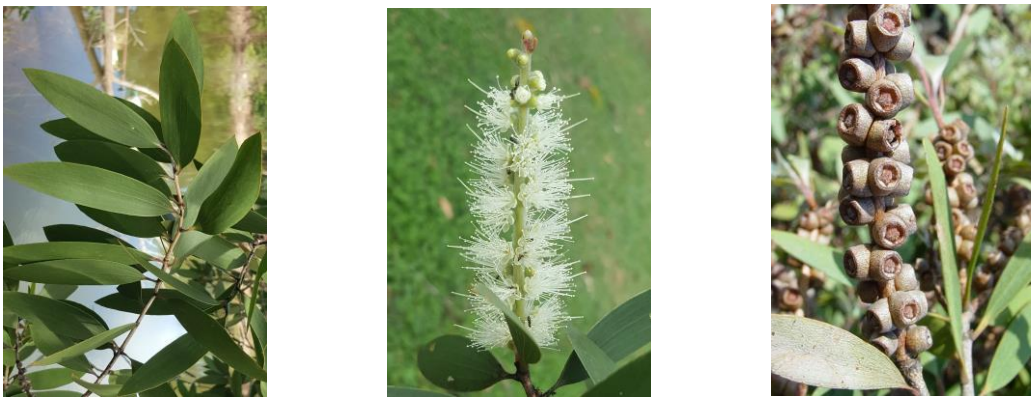
1. Chemicals

All chemicals were purchased from Sigma - Aldrich (USA), Merck (Germany), and Fluka Chemie (Switzerland).

2. Sample Collection and Treatment

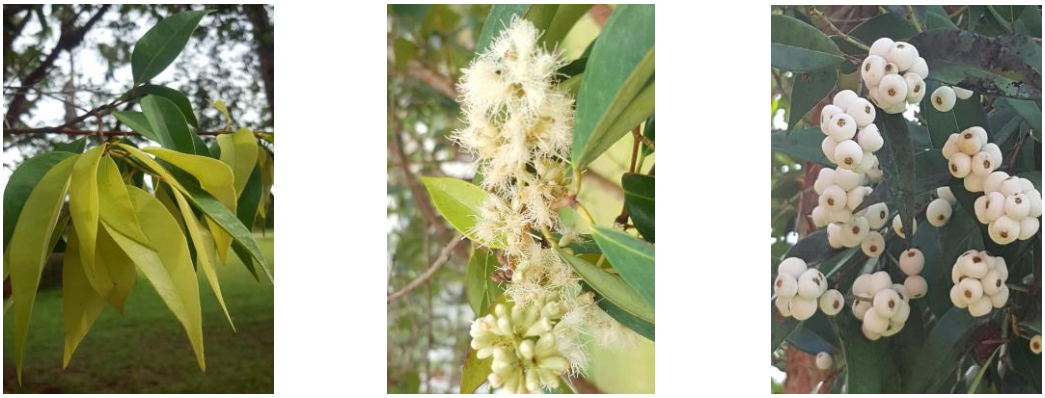
The leaves, flowers and fruits of *Melaleuca leucadendra* and *Syzygium cinerea* were collected from Rajamangala University of Technology Srivijaya, Trang, Thailand in July-September 2017 as shown in Figure 1 and Figure 2. The plants were identified and a voucher specimen were deposited at the Forest Herbarium, Bangkok in Thailand (Specimen BKF no. 194868 and BKF no.194869194870, respectively).

The plant materials were air-dried at room temperature (30 ˚C) for 5 days, after that it was grinded to a uniform powder.



(a) (b) (c)

Figure 1 The parts of *Melaleuca leucadendra* (a) leaves (b) flowers (c) fruits



(a) (b) (c)

Figure 2 The parts of *Syzygium cinerea* (a) leaves (b) flowers (c) fruits

3. Extraction

The crude extracts were prepared by soaking 1,000 g each of the dry powdered plant materials in 4 L of hexane at room temperature for 3 days using maceration technique. The extracts were filtered through a Whatmann filter paper No.1 to give hexane extract. The resultant residue was then soaked in ethyl acetate, ethanol and methanol, respectively. Finally, the 24 supernatants of the different extracts were concentrated using a reduced pressure rotary evaporator with the water bath set at 40 ˚C.

4. Phytochemical Screening

The crude extracts were screened qualitatively for the phytochemical constituents utilizing standard methods of analysis (Harborne, 1998; Trease and Evans, 2002; Sutha, *et al*., 2016).

Anthraquinones determination:

About 200 mg of each crude extracts were boiled with 10 ml of 10% sulphuric acid in a water bath for 5 minutes. The hot mixture was filtered and allowed to cool at room temperature. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and a few drops of 10% ammonia solution was added. The appearance of rose pink color was formed. This showed a positive result for the presence of anthraquinones.

Terpenoids determination:

Salkowski's test: About 200 mg of each crude extracts were dissolved in 2 ml of chloroform. Concentrated sulphuric acid (3 ml) was carefully added to form a layer. The appearance of reddish brown coloration of the interface was formed. This represented a positive result for the presence of terpenoids.

Flavonoids determination:

About 200 mg of each crude extracts weredissolved in 3 ml of 50% ethanol. A small piece of magnesium ribbon was added and the mixture was boiled for few minutes, this was followed by the drop wise addition of concentrated hydrochloric acid. The appearance of reddish pink or dirty brown coloration was formed. This indicated a positive result for the presence of flavonoids.

Saponins determination:

About 200 mg of each crude extracts were added in 5 ml of distilled water in a test tube. The mixture was boiled and filtered through a Whatmann filter paper. The mixture was filtered while hot and allowed to cool. The filtrate was added to 2-3 ml of distilled water and shaken vigorously for 2 minutes and observed for the formation of a stable froth, which appeared after ten minutes. This represented a positive result for the presence of saponins.

Tannins determination:

About 200 mg of each crude extracts were boiled with 5 ml of distilled water for five minutes in a water bath and was filtered. A few drops of 10% ferric chloride were added to the filtrate. The appearance of bluish - black or bluish - green precipitate indicated a positive result for the presence of tannins.

Alkaloids determination:

About 200 mg of each crude extracts were dissolved with 1 ml of 2% sulphuric acid. The mixture was boiled for five minutes in a water bath and then filtered through a Whatmann filter paper. Dragendorff's reagent was added to the filtrate, and the appearance of orange or reddish brown precipitate indicated a positive result for the presence of alkaloids.

RESULTS AND DISCUSSION

By preliminary phytochemical screening of six different chemical compounds such as anthraquinones, terpenoids, flavonoids, saponins, tannins and alkaloids were tested with twenty four different crude extracts.

The preliminary phytochemical studies on hexane, ethyl acetate, ethanol and methanol extracts of *Melaleuca leucadendra* revealed the presence of 61 trials which gave positive results out of 72 trials. The 61 positive results showed the presence of anthraquinones, terpenoids, flavonoids, saponins, tannins and alkaloids. All of the chemical compounds showed maximum presence in ethyl acetate, ethanol and methanol extracts, for all parts of the plant except for the leaves where anthraquinones, saponins and tannins were not found. In hexane extracts for the leaves and fruits, anthraquinones, saponin and tannins were not found, whereas on the flowers, saponin and tannins were not found. The results of qualitative phytochemical analysis on the extracts of *Melaleuca leucadendra* are shown in Table 1.

Table 1 The phytochemical screening of *Melaleuca leucadendra* extracts

Phytochemical	*Melaleuca leucadendra*											
	Leaves				Flowers				Fruits			
	H	EA	E	M	H	EA	E	M	H	EA	E	M
Anthraquinones	-	-	+	+	+	+	+	+	-	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	+	+	-	+	+	+	-	+	+	+
Tannins	-	-	+	+	-	+	+	+	-	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+

Note: H = hexane, EA = ethyl acetate, E = ethanol and M = methanol
+ indicates presence and - indicates absence

The preliminary phytochemical studies on hexane, ethyl acetate, ethanol and methanol extracts of *Syzygium cinerea* revealed the presence of 53 trials which gave positive results out of 72 trials. The 53 positive results showed the presence of anthraquinones, terpenoids, flavonoids, saponins, tannins and alkaloids. All of chemical compounds showed maximum presence in all of parts in ethanol and methanol extracts except in the part of fruits in which anthraquinones were not found. In hexane extracts, no anthraquinones, saponins or tannins, including terpenoids, were found in any parts of the fruits. In ethyl acetate extracts, no anthraquinones or saponins including terpenoids, were found in any parts of leaves. The results of qualitative phytochemical analysis on the extracts of *Syzygium cinerea* are shown in Table 2.

Table 2 The phytochemical screening of *Syzygium cinerea* extracts

Phytochemical	*Syzygium cinerea*											
	Leaves				Flowers				Fruits			
	H	EA	E	M	H	EA	E	M	H	EA	E	M
Anthraquinones	-	-	+	+	-	-	+	+	-	-	-	-
Terpenoids	+	-	+	+	+	+	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	+	+	-	-	+	+	-	-	+	+
Tannins	-	+	+	+	-	+	+	+	-	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+

Note: H = hexane, EA = ethyl acetate, E = ethanol and M = methanol
+ indicates presence and - indicates absence

The phytochemical screening of the leaves flowers and fruits of *Melaleuca leucadendra* and *Syzygium cinerea* in hexane, ethyl acetate, ethanol and methanol extracts showed that two plants generally contain the major secondary metabolites in moderate abundance. These phytochemicals were known to exhibit medicinal physiological activities (Pius *et al.*, 2011).

Anthraquinones are the important class of natural and synthetic compounds with a wide range of applications. Besides their utilization as colorants, anthraquinone derivatives have been used for centuries for medical applications, for example, as laxatives, antimicrobial and anti - flammatory agents include constipation, arthritis, multiple sclerosis and cancer (Enas and Christa, 2016). Furthermore,

anthraquinones showed moderate to strong inhibitors of tyrosinase. This helps digestion, reduces inflammation in arthritis patients and also inhibits the growth of cancer cells.

Terpenoids are considered to be anticancer agent, anti - inflammatory, sedative, insecticidal or cytotoxic activity. Common triterpenes: amyrins, ursolic acid and oleanic acid sesquiterpene like monoterpenes, are major components of many essential oil. The sesquiterpene acts as irritants when applied externally and when consumed internally, their action resembles that of gastrointestinal tract irritant. A number of sesquiterpene lactones have been isolated and they have antimicrobial (particularly antiprotozoal) and neurotoxic action (James, 2012).

Flavonoids are now recognized as possessing an array of bioactivities with several mechanisms relevant to potential reductions in the pathogenesis of chronic diseases such as anti - inflammatory and antioxidant actions as well as alteration of redox - sensitive signal transduction pathways and gene expression (Warra *et al*., 2013). The antioxidant potentials hence could offer protection against heart disease and cancer probably by enhancing the body defense against pathology induced free radicals generation (Enas and Christa, 2016).

Saponins are used industrially in mining and ore separation, in preparation of emulsions for photographic films, and extensively, in cosmetics such as cleansing formula. In addition to their emollient effects, the antifungal and antibacterial properties of saponins are important in cosmetic applications.

Tannins have different functions in that they serve as chelating agents for metals ion, antioxidants in biological systems, and as protein precipitating agents. For human consumption, excess of tannins could be toxicbecause tannins are metal ions chelators and tannin - chelated metal ions are not bioavailable hence could decrease the bioavailability of iron leading to anemia. Furthermore, the previous study had correlated esophageal cancer in humans to regular consumption of certain herbs with high tannin concentration. Thus, the concentration of tannins in the *Melaleuca leucadendra* and *Syzygium cinerea* may not be enough to induce overt toxicity, hence may be appropriate for use in nutraceutical beverage (Pius *et al*., 2011).

Alkaloids are commonly found to have antimicrobial properties against both Gram - positive and Gram-negative bacteria (Johnson *et al*., 2012) and are used functionality in repellence, deterrence, toxicity and growth inhibition by herbivores/predators and in growth inhibition and toxicity by microbes/viruses and as secondary metabolites for UV - protection and nitrogen storage.

Successful determination of biologically active ingredients from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the crude extract, preservative action, inability to cause the extract to become complex or to dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants. The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted (Prashant *et al*., 2011). The solvent effects identified in this study revealed that the most efficient extraction medium for phytochemical constituents

was ethanol and methanol for *Melaleuca leucadendra* and *Syzygium cinerea*.

CONCLUSION

Leaves, flowers and fruits of *Melaleuca leucadendra* and *Syzygium cinerea* contain several chemical constituents. It is also evident that certain parts of *Melaleuca leucadendra* and *Syzygium cinerea* gave a positive test for a particular class of chemical compounds whereas other parts gave negative test for the same class of compounds localization of natural products. *Melaleuca leucadendra* and *Syzygium cinerea* can be a potential source of useful drugs. However, further studies are required to isolate the pure active principle via modern techniques to investigate the extracts for potential pharmacological properties.

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