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TLC Profiling and Phytochemical Screening of Various Extracts of *Ochna integerrima* (Lour.) Merr. from Kog Dong Keng Forest, Thailand

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ABSTRACT

Introduction: *Ochna integerrima* (Lour.) Merr. belongs to family *Ochnaceae,* and it is a traditionally important herb in Southeast Asia. The objective of this study was to investigate the phytochemical compounds of various extracts from six samples. **Methods:** Thin Layer Chromatography (TLC) of the hexane, dichloromethane and 70% ethanol extracts were performed for five important phytochemicals namely, alkaloids, flavonoids, terpene, cardiac glycosides and anthraquinone. **Results:** Terpene was found in all the samples that were extracted from all extracts, while cardiac glycosides and anthraquinone did not occur in all samples that were extracted from all extracts. Flavonoids were observed in all samples that were only extracted with dichloromethane, whereas it was found in some samples from the other extracts. The hexane extract compound was found in alkaloids in *Garcinia cowa* and *Cryptolepis dubia*, while the dichloromethane extract compound was discovered in *Suregada multiflora, Capparis micracantha* and *Salacia chinensis.* All samples extracted with 70% methanol did not contain any alkaloid compounds. Terpene was found in the hexane extracted compounds. **Conclusions:** These findings suggested that *Ochna integerrima* (Lour.) Merr. is a potent source of medicinal phytochemical compounds that could lead to its use as medicinal products.

Key words: *Ochna integerrima* (Lour.) Merr., Phytochemical compounds, TLC, Flavonoids.

INTRODUCTION

Ochna integerrima (Lour.) Merr. belongs to genus *Ochna* (family *Ochnaceae*). It is native to Southern Asia, Indochina region and Malay Peninsula, namely India, Myanmar and Pakistan. In Thailand the genus is normally found in mixed forest, deciduous forest, dry evergreen forest and the coast at heights up to 1200 meters. The peel is used for treating dysentery and bloody diarrhea. *Ochna* L. has 85 varieties that were found in Africa, while there were four varieties in Asia. In a traditional Thai medicine textbook, *Ochna integerrima* (Lour.) Merr. was boiled with water to nourish the body or mixed with other herbs for restoral of the body. The Muser hill tribe ferment parts of the roots *Ochna integerrima* (Lour.) Merr. with alcohol (white liquor or rice whisky) or boil it with water for restoral of the body. Moreover, it has shown antimicrobial activity towards *Staphylococcus epidermidis* (Sritubtim, Krapeedeang and Wongsa, 2014).[1] Many researchers of natural product have investigated and developed newer and safer drugs from plants based on their use in traditional systems of medicine.[2,3] The phytochemical compounds in the plant can indicate its medicinal potential. Flavanoids are one of the phenolic compounds that play a role with antioxidant, anti-inflammatory, anti-allergic and anticancer activities. Alkaloids show antiarrhythmic, antimicrobial, analgesic and anticancer activities. Steroids have been observed as signaling molecules and they are important against cardio tonic activity.[4]

The traditional herb *Ochna integerrima* (Lour.) Merr. has been used to nourish the body for a long time. However, it has not been assessed for its bioactive compounds. Thus, this study investigated the bioactive compounds from various extracts of *Ochna integerrima* (Lour.) Merr, and to compare them to five samples from the same forest. Knowledge of the bioactive compounds of plants is important. The information provided by this research may be used for herb management and conservation, and could help developed new products in the future.

MATERIALS AND METHODS

Sample collection

The plant samples of *Ochna integerrima* (Lour.) Merr, *Suregada multiflora*, *Garcinia cowa*, *Capparis micracantha*, *Cryptolepis dubia* and *Salacia chinensis* were collected from Kog Dong Keng Forest, Nadoon District, Mahasarakham Province and the community forest of Don Ya Nang Village, Phon Charoen District, Bueng Kan Province. Samples were washed, air dried, homogenised to a fine powder and then stored in aluminum bags.

Sample extraction

The samples (5 g dry basis) were extracted with 25 ml of 70% ethanol and then sonicated for 30 min. The extract was filtered through filter paper (Whatman™). The extract was filtered using the partition method with 100 ml of hexane and then this extract was extracted three times. The extract was separated into two layers and then the layer of the hexane solution was evaporated by rotary evaporator at 60 °C, and then the dried extract was added to 10 volumes. The 70% ethanol layer was extracted using the partition method with 100 ml dichloromethane

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and this was evaporated with a rotary evaporator at 60 °C, and then the dried extract was added to 10 volumes. The 70% ethanol extract was evaporated with a rotary evaporator at 60 °C, and then the dried extracted was add to 10 volumes.

Thin layer chromatography (TLC) method

The three extracts were analyzed with Thin Layer Chromatography (TLC) using silica gel 60 as the stationary phase. The TLC profiling was determined as described by a previous study.[5] The sample was evaluated with several reagents (Dragendorf's reagent, anisal-sulfuric acid reagent, 5% KOH reagent, Kedde's reagent, phosphomolypdic acid reagent, natural product reagent) to enable the analysis of the group. The samples came from six plants *Ochna integerrima* (Lour.) Merr. as well as *Suregada multiflora*, *Garcinia cowa*, *Capparis micracantha*, *Cryptolepis dubia* and *Salacia chinensis*. Three solvents were used for comparison of the extraction efficiency: (1) hexane, (2) dichloromethane and (3) 70% ethanol. Different extracts of the six herbs were obtained by extraction with various solvents of increasing polarity. TLC was performed for alkaloids, flavanoids, anisaldehyde, terpenoids, cardiac glycosides and phenolics.

RESULTS

TLC profiling images of hexane, chloroform and 70% ethanol extracts are shown in Figure 1. The TLC screening of the bioactive compounds from the extracted samples by hexane are presented in Table 1. The suitable ratio for the mobile phase using the solvent system of hexane and ethyl acetate was 75:25. Out of the six samples treated with Dragendorff's reagent to test for alkaloids, *Ochna integerrima* (Lour) Merr. tested negative.

As it was not present as a red-orange in the TLC, while five samples were detected with this colour that showed a positive test. Terpenoids are the derivative of volatile compounds, and six samples tested positive for the presence of terpenoids with anisaldehyde in H_2SO_4. It was confirmed by the blue, purple, pink or green colours of the spot after application of anisaldehyde in an H_2SO_4 application. Both *Garcinia cowa* and *Cryptolepis dubia* tested positive for the presence of flavonoids, while *Capparis micracantha* and *Ochna integerrima* (Lour) Merr. tested positive for the presence of flavonoids. The natural product reagent tests were shown by an orange colour on the TLC plate. A blue colour was shown on the TLC after applying the natural product

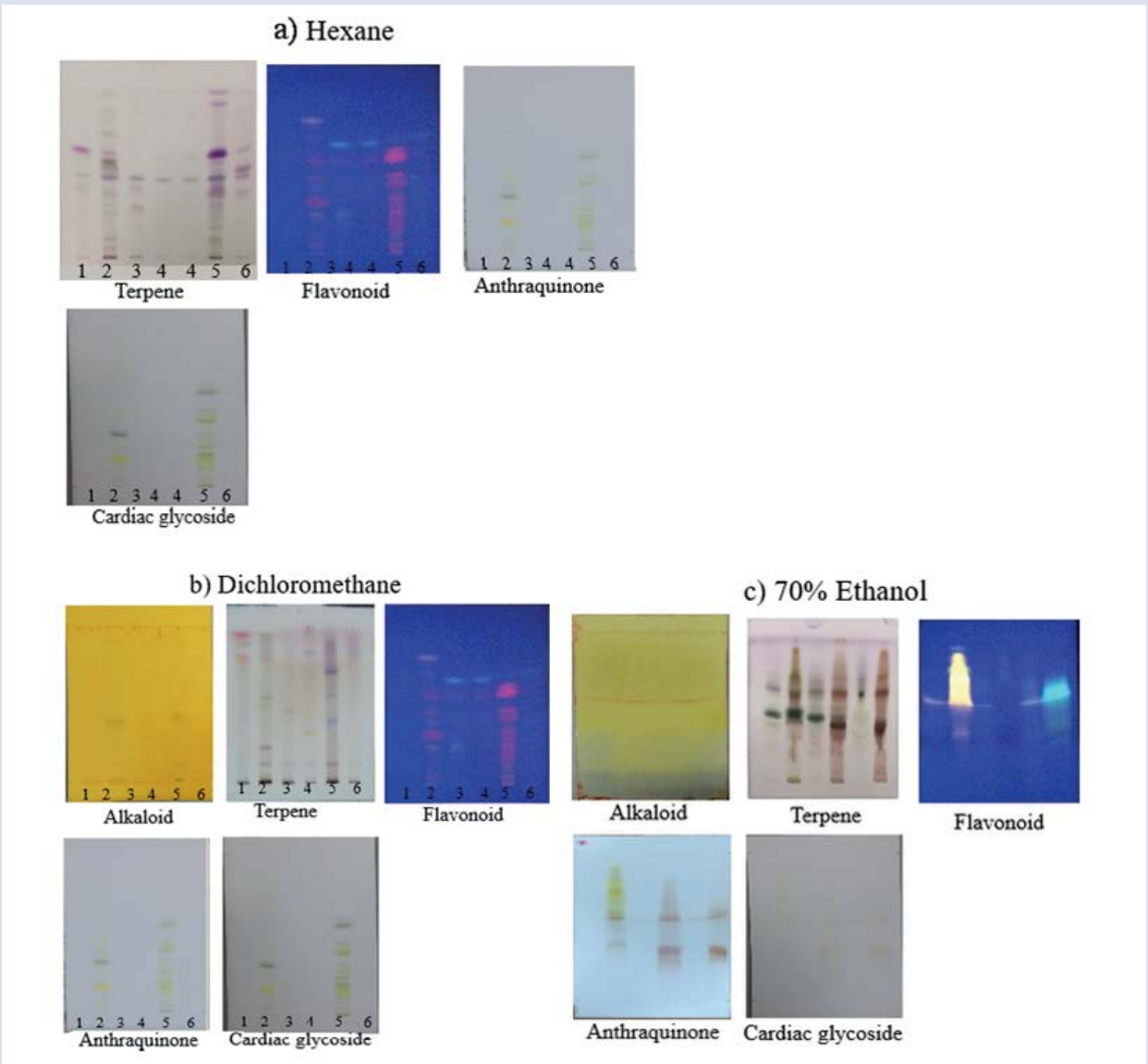

Figure 1: TLC profiling images of (a) hexane, (b) dichloromethane and (c) 70% ethanol extracts for 1) *Ochna integerrima* (Lour) Merr., 2) *Suregada multiflora*, 3) *Garcinia cowa*, 4) *Capparis*, 5) *Cryptolepis dubia* and 6) *Salacia chinensis.*

Table 1: Phytochemical screening of hexane extracts of six herbs.

Sample	Alkaloid	Flavonoid	Terpene	Cardiac glycoside	Anthraquinone
Ochna integerrima (Lour) Merr.	-	-	+	-	-
Suregada multiflora	-	-	+	-	-
Garcinia cowa	+	+	+	-	-
Capparis micracantha	-	-	+	-	-
Cryptolepis dubia	+	+	+	-	-
Salacia chinensis	-	-	+	-	-

(+) mean positive test and (–) mean negative test

Table 2: Phytochemical screening of dichloromethane extracts of six herbs.

Sample	Alkaloid	Flavonoid	Terpene	Cardiac glycoside	Anthraquinone
Ochna integerrima (Lour) Merr.	+	+	+	-	-
Suregada multiflora	+	+	+	-	-
Garcinia cowa	-	+	+	-	-
Capparis micracantha	+	+	+	-	-
Cryptolepis dubia	-	+	+	-	-
Salacia chinensis	+	+	+	-	-

(+) mean positive test and (–) mean negative test

Table 3: Phytochemical screening of 70% ethanol extracts of six herbs.

Sample	Alkaloid	Flavonoid	Terpene	Cardiac glycoside	Anthraquinone
Ochna integerrima (Lour) Merr.	-	+	+	-	-
Suregada multiflora	-	+	+	-	-
Garcinia cowa	-	+	+	-	-
Capparis micracantha	-	-	+	-	-
Cryptolepis dubia	-	-	+	-	-
Salacia chinensis	-	-	+	-	-

(+) mean positive test and (–) mean negative test

reagent for phenolics. Anthraquinone related compounds were tested with a 3% KOH application, using hexane:ethyl acetate (75:25) as the mobile phase for the TLC analysis. All six samples tested negative for anthraquinone related compounds. These samples presented a range of colours from pink to red. The result showed that the six herbs did not contain anthraquinone compounds or cardiac glycoside compounds that were tested negative by Kedde's reagent for all samples, as it did not show a blue or purple on the TLC after Kedde's reagent application.

The suitable ratio for using the solvent system of dichloromethane and acetone was 4:3. The TLC screening of the bioactive compounds from the extracted samples ratio using the solvent system dichloromethane and acetone (4:3) are presented in Table 2. Out of the four samples treated with Dragendorff's reagent to test for alkaloids, *Suregada multiflora, Capparis micracantha, Ochna integerrima* (Lour)Merr. and *Salacia chinensis* tested positive. All six samples tested positive for the presence of terpenoids with anisaldehyde sulfuric reagent. The result was confirmed by the blue, purple, pink or green colour of the spot after FeCl3 application. All six samples tested positive for the presence of flavonoids, while *Cryptolepis dubia* also tested positive for the presence of phenolics. The TLC plate of all six samples were not observed for the range of pink to red colour after 3% KOH reagent application. The result showed that the six herbs did not contain the anthraquinone compounds. Cardiac glycoside compounds were tested negative by Kedde's reagent for all samples.

The ratio of the solvent system 1-butanol: methanol: water: formic acid was 4:2:2:0.25. The TLC screening of the bioactive compounds from the 70% ethanol extract are presented in Table 3. Alkaloid compounds were found in four samples: *Suregada multiflora*, *Capparis micracantha*, *Ochna integerrima* (Lour) Merr. and *Salacia chinensis,* which tested positive. All six samples tested positive for the presence of terpenoids and terpenoid-related compounds.

Terpenoids are derivatives of volatile compounds. All six samples tested positive for the presence of flavonoids while, *Cryptolepis dubia* also tested positive for the presence of phenolics. A positive result was shown by a pink to red color on the TLC plate. All six samples tested negative for anthraquinone related compounds. Cardiac glycoside compounds gave a negative result from Kedde's reagent for all samples. The positive result was shown by purple or blue color on the TLC plate. The result demonstrated that all samples did not contain cardiac glycoside compounds.

The TLC profiling of all six herbs revealed the presence of different metabolites, such as alkaloids, flavonoids, phenolics, anthraquinone and cardiac glycosides. This research showed that those six herbs were found to contain three compounds, namely alkaloids, terpenoids and flavonoids. These three compounds were dissolved with different solvents, such as both *Garcinia cowa* and *Cryptolepis dubia*were that contained alkaloids as shown by the hexane extract, while *Suregada multiflora, Capparis micracantha, Ochna integerrima* (Lour) Merr. and *Salacia chinensis* contained alkaloids as shown by the dichloromethane extract. None of the samples gave positive tests with 70% ethanol. Terpenoid is a derivative component of saponin and volatile compound in the terpene group which were found in volatile compounds and were extracted by hexane and dichloromethane, while saponin compounds were exhibited by the 70% ethanol extract. There was terpene in three extracts, namely hexane, dichloromethane and 70% ethanol for all samples. The result indicated that all six herbs exhibited bioactive phytochemical compounds.

DISCUSSION

Some or all of the parts of the plants synthesized various secondary metabolites that could have antimicrobial and antioxidant properties as shown by the results of the phytochemical TLC screening. This result

presents various important phytochemical differences in the extracts of the plants. These bioactive phytochemicals from six herbs that are medicinal plants could have therapeutic potential and be useful for the treatment of many diseases. Bioactive phytochemical compounds from plants have been found that have medicinally significance roles that produce characteristic physiological actions on humans.[6] Different solvents can show the presence of different phytochemicals. The variant polarity of the solvents was used to receive the phytochemicals. Many of the phytochemicals, namely alkaloids, flavonoids and terpene, were found in the hexane, dichloromethane and 70% ethanol extracts. Terpenoids are credited with analgesic and anti-inflammatory activities, while flavonoids have been reported to be responsible for many useful characteristic properties, including antioxidant, antimicrobial, anti-inflammatory, antiallergic and cytotoxic antitumor activities.[7] Medicinal plants with bioactive compounds have been used in the treatment of many diseases.[8]

Thin layer chromatography is usually used for bioactive compound identification. It was used to determine the phytochemicals from plants.[9-11] In this study, the TLC profiling of all the plant extracts indicated the presence of different metabolites, such as alkaloids, flavonoids, terpene, cardiac glycoside and anthraquinone. Among the three solvents (hexane, dichloromethane and 70% ethanol extract), dichloromethane was found to be effective in extracting the maximum number of secondary metabolites in all samples. Different solvent extracts of the phytochemicals provided further information about selecting a particular solvent system from their polarity and isolation of any compound from the plant extracts using chromatographic techniques.[5]

CONCLUSION

The results of the phytochemical analysis suggested that all samples produced many secondary metabolites of bioactive compounds. TLC profiling further confirmed the presence of alkaloids, flavonoids, terpene, cardiac glycosides and anthraquianone, which can be dissolved in different solvents. The hexane extract compound found alkaloids in *Garcinia cowa* and *Cryptolepis dubia*, while the dichloromethane extract compound was discovered in *Suregada multiflora, Capparis micracantha* and *Salacia chinensis*. All samples extracted with 70% methanol did not show any alkaloid compounds. Terpene was found in the hexane extracted compounds, thus indicating that all samples consisted of volatile compounds and saponins. The plants observed showed many phytochemicals that can be used to reduce the risk from many diseases. Therefore, the plants can be used to develop medical products or as ingredients in functional food products. This present study confirms that there were important phytochemicals in the unexplored plant *Ochna integerrima* (Lour) Merr and five herbs from Kog Dong Keng Forest, Nadoon District, Mahasarakham Province and the community forest of Don Ya Nang Village, Phon Charoen District, Bueng Kan Province.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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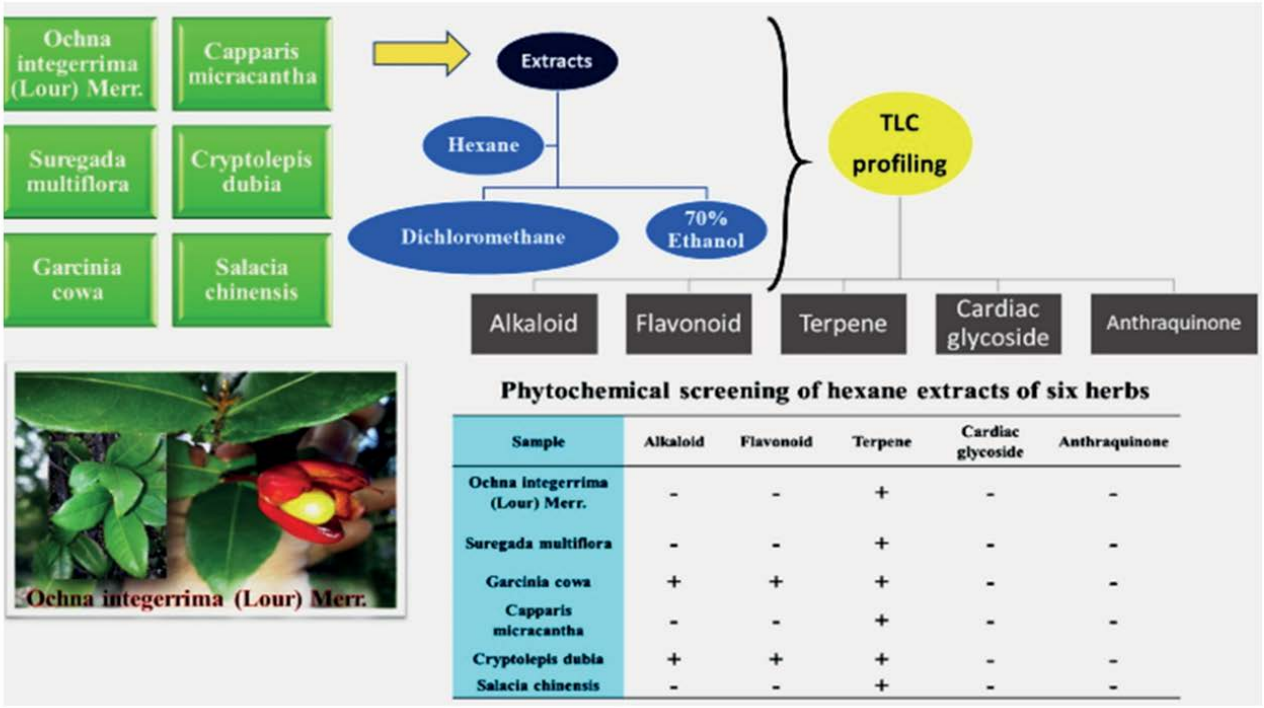
GRAPHICAL ABSTRACT



Sample	Alkaloid	Flavonoid	Terpene	Cardiac glycoside	Anthraquinone
Ochna integerrima (Lour) Merr.	-	-	+	-	-
Suregada multiflora	-	-	+	-	-
Garcinia cowa	+	+	+	-	-
Capparis micracantha	-	-	+	-	-
Cryptolepis dubia	+	+	+	-	-
Salacia chinensis	-	-	+	-	-

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