Original article

THAI BULLETIN OF PHARMACEUTICAL SCIENCES
ISSN 2586-8659 (online)

EXTRACTION AND ANALYSES OF PHYTOCHEMICAL COMPOUNDS FROM *CITRUS HYSTRIX* PEELS FOR MOLLUSCICIDAL ACTIVITIES

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ABSTRACT

The objective of this work was to screen, identify, and quantify the phytocompounds contained within *Citrus hystrix* peel extracts, in different extraction solvents, for molluscicidal activities. The peel of *C. hystrix* was extracted through the process of maceration with methanol. The phytochemical compounds found within the crude methanolic extract were further isolated through fractional partition with various solvents including hexane, dichloromethane, and butanol. Using thin-layer chromatography, phytochemical screenings for triterpenes, steroids, phenolic compounds, and alkaloids were carried out for each extract/fraction. The stability of the extracts/fractions were studied under static storage and accelerated conditions. The findings revealed that methanol was a suitable solvent for primary extraction, due to the high number and yield of compounds found, including non-polar, medium polar and high polar substances. After fractional extraction, non-polar compounds retained within hexane layer whereas both polar and medium polar substances were detected in methanol extract, dichloromethane and butanol fractions. The extracts were stable in normal storage conditions. Thus, this investigation could be of use for those conducting further research into the pharmacological activities of compounds contained within *C. hystrix* peel.

Keywords: *Citrus hystrix*, *Pomacea canaliculata*, phytochemical pesticide, molluscicidal effect

Received: 15 August 2022; Revised: 21 September 2022; Accepted: 21 September 2022

Introduction

Pomacea canaliculata L., also known as golden apple snail (GAS), is considered a major, wide-spreading invasive pest, that poses a threat to agricultural crops. The golden apple snail is known to destroy rice crops due to its high reproduction rate, resistance to drought, and mobility through water flow.[1,2] Several efforts are made to control the GAS, including mechanical, biological, and chemical approaches. The mechanical method is an effective, cost-saving, and non-toxic way of removing GAS. This can be done by manually collecting, trapping, and luring the pest and its eggs for further elimination. The biological approach can be done by eliminating GAS through the introduction of farm animals such as ducks and certain types of pelicans that consume GAS as their food. Lastly, the chemical approach involves the use of copper sulfate, 20% emulsifiable concentrate formulation of niclosamide (Bayluscide®), and metaldehyde.[3-7]

One of the simplest ways to get rid of these pests is the use of chemical pesticides. These chemicals are usually toxic to humans and the environment, and unfortunately, are also likely to contaminate farm products, ecosystems, the environment, soil, and water.[8,9] The harms of these chemicals has been recognized and led to the seeking of alternative substances including plants and herbs. According to the literature, natural plants including tobacco (*Nicotiana tabacum*), ginger (*Zingiber officinale*), Siamese neem tree (*Azadirachta indica*), and greater galangal (*Alpinia galanga*) were beneficial as natural insecticides.[10-13] However, the use of other plants, herbs, or other natural compounds has not yet been widely reported.[14]

One such group of important and interesting substances is limonoids - phytochemicals of the triterpenoid class which are abundant in Citrus (*Citrus* spp.). Limonoids have been shown to offer antifeedant activity against insects, as well as other qualities including growth regulation, antibacterial and anticarcinogenic properties, antitumorigenic activities, and the induction of apoptosis. Moreover, compared to other genera of plants, citrus plants have been reported to be toxic to mollusks without harmful effects to aquatic lives.[15-18] In Thailand, the cultivation of medicinal plants including fruits in this genus for human use and consumption is widely adopted. Nevertheless, peels of citrus fruit, especially *Citrus hystrix*, have been primarily used in combination with other herbs to make fermented concoctions according to local wisdom to combat GAS. *Citrus* spp. are also known to contain several flavonoids, i.e., naringin and hesperidin with reported biological activities.[19-21]

Previous research has suggested the use of plants within the Citrus family including *Citrus hystrix*, leech lime, or kaffir lime, to control GAS.[22,23] It was conveyed that the enzymes, glycogens, proteins, and other biochemicals found in the plant exhibited molluscicidal activity.[24] In this work, the phytocompounds contained within *C. hystrix* peels were extracted and then further isolated. Various phytochemical screenings were performed on these extracts Furthermore, the stability to hot and cold environments of the extracts was also evaluated. This research could provide an insight into compounds that could provide molluscicidal activity and support further investigations into the use of *C. hystrix* peel extracts in the fight against GAS, which would assist in the reduction of chemical use in the future.

Materials and Methods

Materials

Citrus hystrix was obtained from Nakhon Pathom province, Thailand. Methanol, butanol, dichloromethane (DCM), hexane, sulfuric acid, hydrochloric acid, potassium hydroxide, glacial acetic acid, and ethyl acetate were purchased from Merck & Co. (Darmstadt, Germany). Rutin, 3,5-dinitrobenzoic acid, limonin, hesperidin, naringin, and nobiletin were bought from Sigma Aldrich (St. Louis, MO, USA). Other chemicals and reagents were used as received.

Extraction from *C. hystrix* peel

The fruit of *C. hystrix* was carefully washed in a washbasin. The pericarp was peeled out and cut into small pieces. The peels were then dried in a hot air oven at 50ºC. Two hundred grams of dried *C. hystrix* peels were subsequently transferred into an Erlenmeyer flask and macerated twice in 2 liters of methanol. Subsequently, each methanol extraction was combined, filtered, and the solvent was removed via a rotary evaporator to yield crude methanolic extracts. The crude extracts were stored in a desiccator until the time of use.

Thereafter, the compounds of crude methanolic extracts were further separated using the various polarities of organic solvents by employing the solvent partition technique. The extraction was performed between aqueous and organic solvents of different polarities including, hexane, DCM, and butanol. Each of the extraction solvents were removed *in vacuo* to yield hexane, DCM, and butanol fractions, respectively. The aqueous layers from the last extraction were also evaporated on a hot bath to collect the aqueous fraction. These extracts were kept in a desiccator until required. The %yield of each extract/fraction was calculated using equation 1.

$$\%\text{Yield} = \frac{\text{weight of dried crude extract}}{\text{weight of dried } \textit{C. hystrix}} \times 100 \text{ -------(1)}$$

Qualitative phytochemical screening of *C. hystrix* peel

Phytochemical screening[25] was performed to determine the bioactive phytocompounds contained within the *C. hystrix* peel extracts. Each extract was prepared at an extract concentration of 10 mg/mL in ethanol. The tests for triterpenes, steroids, phenolic compounds, and alkaloids were performed as follows.

Screening of triterpenes and steroids

The presence of any triterpenes and steroids contained within the *C. hystrix* peel extracts was determined by conducting the Liebermann-Burchard test (LB test). In brief, an aliquot of the extract was placed into an evaporation dish and evaporated until dry on a water bath. Then, a few drops of acetic anhydride were added and the solution stirred with a glass rod. A small amount of concentrated sulfuric acid was then (gently) poured into the solution. The presence of triterpenes in the extract was observed by the appearance of a purplish color. The emergence of a greenish-blue color indicated the presence of steroidal structures. The presence of both colors in a layer conveyed the presence of both triterpenes and steroids.

Glycosides in the *C. hystrix* peel extracts were examined by Molisch's test. Briefly, the extract solution (2 mL) was prepared in a test tube. Then, a few drops of the Molisch reagent were added to the extract solution, followed by 1 mL of concentrated sulfuric acid which was slowly added along the inner side of the test tube. Without agitation of the test tube, the formation of a purple ring at the interface indicated the presence of glycosides in the extract.

The presence of any saponins in the *C. hystrix* peel extracts was determined by the foam formation test. One milliliter of the extract solution was diluted with 20 mL distilled water, contained in a 50-mL cylinder. The mixture was shaken vigorously for 15 minutes. The formation of a stable foam indicated the presence of saponins.

The presence of cardiac glycosides in the *C. hystrix* peel extracts must fulfil positive results for both Liebermann-Burchard test indicative of the presence of steroidal nucleus, and Kedde's test indicative of the presence of unsaturated lactones ring. by. In the Kedde's test, the test solution was mixed with Kedde reagent (2% 3,5-dinitrobenzoic acid in methanol and 7.5% potassium hydroxide (1:1 v/v) to yield a blue-violet color. Furthermore, deoxy-sugar of cardiac glycosides must show positive result by Keller-Kiliani test. In the Keller-Kiliani test, the test solution was treated with a few drops of ferric chloride solution, followed by the gentle addition of a layer of concentrated sulfuric acid. The appearance of a reddish-brown ring at the interface indicated the presence of a deoxy-sugar, characteristic of cardenolides.

Screening of phenolic compounds

Various phenolic compounds can be found in plant extracts leading to the possibility of different color development, such as green, blue, brown, or black after reacting with ferric chloride ($FeCl_3$) test solution. Hence, various tests were also conducted to differentiate each phenolic compound as followed.

The presence of flavonoids in an extract was determined by using the Cyanidin test. Magnesium pieces and concentrated hydrochloric acid were added to the extract solution. The appearance of a pink, orange, or red color in the solution indicated the presence of flavonoids.

The presence of tannins in the extract was verified by the Gelatin test. Gelatin aqueous solution was added to the extract solution. The formation of white precipitates indicated the presence of tannins.

The presence of anthraquinones in an extract was checked by Borntrager's test. The extract solution was boiled in 10% sulfuric acid and filtered. To the cooled filtrate, an equivalent volume of ethyl acetate was added and the mixture was shaken to partition the two immiscible liquid solvents. After the separation of the organic and aqueous layers, the aqueous layer was removed and discarded. Thereafter, 10% ammonia solution was added to the ethyl acetate phase. The mixture was shaken and left to separate into two layers. The presence of pink, orange, or red in the basic layer and yellow to colorless in the organic layer, indicated the presence of anthraquinones.

Other phenolic compounds did not give a positive result on any of the aforementioned tests. They were needed to be confirmed using other phenolic group examinations. For example, some phenolic, glycosidic compound gave a positive result to Molisch's test.

Screening of alkaloids

The presence of alkaloids in the extract was determined by carrying out a reaction test using Dragendorff's reagent. The prepared extract solution was evaporated to dryness on a water bath. A few drops of diluted hydrochloric acid solution were added into the residue, and then a few drops of Dragendorff's reagent were added. The presence of orange-reddish brown precipitates would indicate the presence of alkaloid structures.

Qualitative determination of phytochemical compounds in the extracts using thin-layer chromatography (TLC)

The presence of various components within the extracts of *C. hystrix* was also confirmed by thin-layer chromatography (TLC). The stationary phase was flat support coated with silica gel GF254, while the mobile phase was a mixture of ethyl acetate, methanol, and water at a ratio of 100:13.5:10. The chromatogram was observed under UV light with wavelengths of 254 and 365 nm. Further tests were also performed by spraying the TLC with anisaldehyde-sulfuric acid spraying reagent and heated at 110 ºC.

Quantification of flavonoids

The flavonoid content of *C. hystrix* extracts was quantified by high-performance liquid chromatography (HPLC) following the protocol described previously.[26] Hesperidin, naringin, and nobiletin were used as flavonoid standards. Briefly, 1 gram of the dried extract/fraction was mixed with 50 mL of methanol and filtered through a 0.45-µm nylon filter. HPLC separation was performed on a reverse-phase column (Symmetry C18, 3.9 x 150 mm). The mobile phase consisted of 0.01 M phosphoric acid (solvent A) and methanol (solvent B). Elution was achieved with a gradient flow; 0-55 minutes: 70-55% A; 55-95 minutes: 55-0% A and 95-100 minutes: 100% B. The flow rate was set at 0.6 mL/minute. The temperature of the column was maintained at 40°C. The contents of the flavonoids were analyzed by using a UV detector at 285 nm. Under these conditions, retention times for naringin, hesperidin, and nobiletin were 17.5, 20.8, and 71.3 minutes, respectively. Standard curves of naringin, hesperidin and nobiletin were constructed at the concentration range of

5-2500 ppm. The flavonoid substances of the extracts were identified and quantified, relative to the standard retention times and peak areas.

Quantification of limonin

Limonin is a limonoid, a bitter substance found in Citrus and other plants. Chemically, it is a member of the class of compounds known as furanolactones, related to triterpene derivatives.

The limonin content of *C. hystrix* extracts was determined by HPLC using the procedure reported in a previous study.[27] Accurately weighed (1 gram) extract/fraction was dissolved in 50 mL of methanol and filtered through a membrane. The analysis was performed using a C18 column (Symmetry C18, 4.6 x 150 mm). Elution was performed isocratically using a mobile phase consisting of methanol, acetonitrile, and water (1:37:62 v/v/v) with a flow rate of 1 mL/minute. The total running time of each injection was 30 minutes. The column was kept at an ambient temperature. A UV detector was used with a fixed wavelength of 210 nm. The retention time of limonin was 22.8 minutes. The standard curve of limonin was prepared at a concentration range of 5-625 ppm. The limonin content was calculated according to the peak area of the sample, relative to the limonin standard.

Stability study

The stability of the extracts was evaluated by analyzing their physical and chemical properties. The physical characteristics of the freshly prepared extract, including appearance, color, odor, and pH, were compared with those that had been stored under static and accelerated storage conditions.[28] The static storage conditions were: 8 days with light exposure at 25ºC, followed by 8 days in the dark at 25ºC. The accelerated conditions involved heating and cooling cycles where the extracts were alternately kept at 45ºC for 24 hours and then at 4ºC for 24 hours repeatedly, over 4 cycles. Chemical stability was determined using HPLC, following the aforementioned procedures.

Statistical analysis

Numerical data were reported in mean ± standard deviation (SD). The data were tested for statistical significance using one-way ANOVA followed by Dunnett's post-hoc test. Significant differences were reported at $p < 0.05$.

Results and Discussion

***C. hystrix* extraction**

The phytocompounds contained within *C. hystrix* peel were extracted through the process of maceration with methanol. Then, the methanolic extract was further fractionated using organic solvents with different polarities including hexane, dichloromethane (DCM) and butanol, using the solvent partition method. The percentage yields of each dried extract are presented in Table 1. It was found that the %yield of crude methanolic extract was quite high relative to the weight of dried fruit skin, indicating that methanol was a suitable solvent for the primary extraction of *C. hystrix* peel. The % yields of the subsequent extractions were in the order: aqueous fraction > butanol fraction > DCM fraction > hexane fraction, as shown in Table 1.

Qualitative phytochemical screening of *C. hystrix* peel extracts

Phytochemical screening was conducted to identify the phytocompounds present within the *C. hystrix* peel extracts. As shown in Table 2, triterpenes and/or steroids were found in all the organic solvent extracts. More precisely, observation of the color shade test results showed that steroidal saponins were most often found in the hexane and DCM extracts. Their presence was indicated by the greenish color that emerged when conducting the Liebermann-Burchard test (LB test) and from the foam test results. Similarly, the DCM extract also contained triterpenoid saponins, that resulted in a purple color appearing when the LB test was conducted, and a positive foam test. Phenolic structured substances were detected in both aqueous and organic solvents; therefore, it is likely

that flavonoid glycosides were present in these extracts, except for the hexane fraction which tested negative on the cyanidin test. Tests for alkaloids only returned positive results in the crude methanolic extract that was subjected to the Dragendorff's test. The crude methanolic extract was found to contain flavonoid glycosides, steroidal/triterpenoid saponins, and alkaloids. The hexane fraction only contained steroidal saponins. The DCM fraction contained both steroidal, triterpenoid saponins and flavonoid glycosides. The butanol fraction contained flavonoid glycosides and triterpene structures. Lastly, the aqueous fraction only contained flavonoid glycosides.

Table 1 %Yield (of dry weight) of *C. hystrix* extracts/fractions from different solvents

Extracts	**% yield (w/w)**
Methanolic extract	68.86 ± 7.31
Hexane fraction	4.75 ± 0.76
DCM fraction	16.87 ± 1.89
Butanol fraction	18.11 ± 1.03
Aqueous fraction	23.31 ± 2.31

Table 2 Qualitative phytochemical screening of phytocompounds of different *C. hystrix* extracts

Test method ***(Test compound)***	**Methanolic extract**	**Hexane fraction**	**DCM fraction**	**Butanol fraction**	**Aqueous fraction**
Lieburmann-Burchard test *(Triterpenes/Steroids)*	+ *(Purple/Green)*	+ *(Green)*	+ *(Purple/Green)*	+ *(Purple)*	–
Molisch's test *(Glycosides)*	+	+	+	+	+
Foam test *(Saponins)*	+	+	+	–	–
Kedde's test *(Unsaturated lactone ring)*	–	–	–	–	–
$FeCl_3$ test *(Phenols)*	+	+	+	+	+
Cyanidin test *(Flavonoids)*	+	–	+	+	+
2% Gelatin Solution *(Tannins)*	–	–	–	–	–
Borntrager's test *(Anthraquinones)*	–	–	–	–	–
Dragendorff's reagent *(Alkaloids)*	+	–	–	–	–

Qualitative determination of phytochemical compounds in the extracts using thin-layer chromatography (TLC)

The *C. hystrix* peel extracts were further investigated for their phytochemical components using TLC. The TLC chromatograms were observed under UV light at 254 and 365 nm. The compounds were characterized through the TLC fingerprint as presented in Figure 1. It was observed that the compounds in the aqueous fraction of *C. hystrix* could not be identified using these chromatographic conditions and were excluded from further TLC analysis. According to the TLC fingerprint in Figure 1, various types of substances were found in the methanol extract, DCM, and butanol fractions, as different bands appeared on the TLC chromatogram. Hexane fraction gave only a few bands, with the higher retention factor (Rf) occurring near the solvent front, indicating the existence of non-polar compounds such as chlorophyll. Furthermore, rutin, a commonly found flavonoid glycoside in plants, was added to the TLC plate as a marker (figure 2-3). In figure 2 and 3 standard flavonoid (rutin) was run in parallel to all extracts, except aqueous fraction, and was detected in the chromatogram under UV light at 254 and 365 nm (figure 2). The TLC chromatogram in figure 3 was derivatized with anisaldehyde-sulfuric acid reagent before being examined under white light and UV light at 365 nm. The results in figures 2 and 3 shows many bands of various constituents, especially in the methanolic extract and DCM fraction of *C. hystrix* peel, but no band appeared to have the same Rf value as that of rutin. Derivatization of TLC chromatogram with anisaldehyde-sulfuric acid reagent enabled the detection of bands of terpenoids, saponins, sterols and most lipophilic compounds[29] which was in accordance with the phytochemical screening analysis. The chromatograms showed that various types of phytocompounds were contained within the *C. hystrix* peel extracts. It was found that the hexane fraction was composed of non-polar substances, while DCM and butanol fractions contained both polar and medium-polar compounds. Therefore, these phytocompounds may act as bioactive components resulting in the molluscicidal activity of *C. hystrix* against GAS as reported in earlier literature.[16]

Quantification flavonoid and limonin and the stability study

Previous studies[22-24] showed that *C. hystrix* peel methanol extract, including the hexane and dichloromethane fractions of the methanolic extract, were found to be toxic to GAS, suggesting that *C. hystrix* methanol extract and the hexane and dichloromethane fractions may contain molluscicidal substances. We then sought after the quantity of compounds that could be related to the molluscicidal

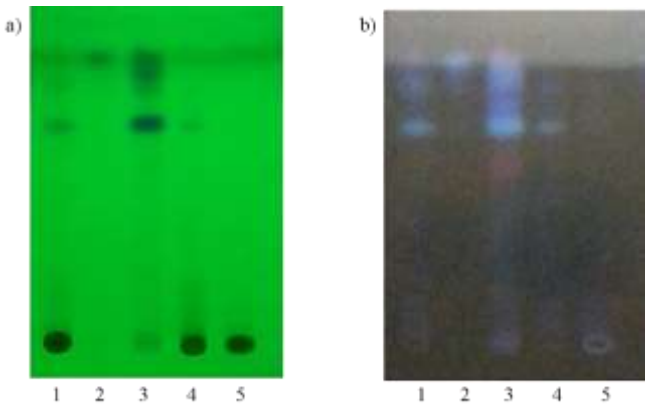

Figure 1 TLC chromatograms of (1) methonolic extract, (2) hexane fraction, (3) DCM fraction, (4) butanol fraction and (5) aqueous fraction of *C. hystrix* peel observed under a) 254 nm and b) 365 nm.

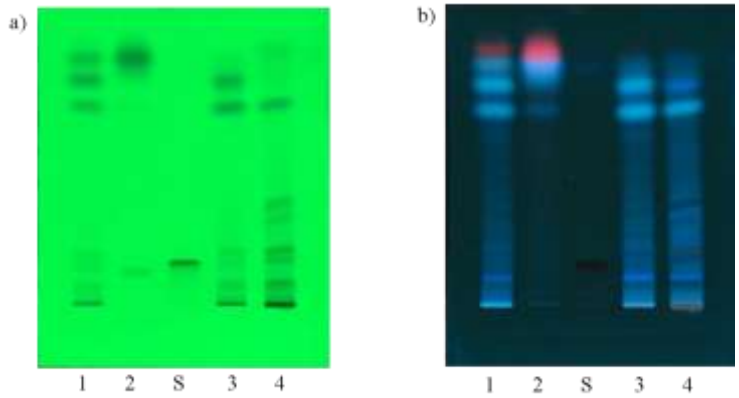

Figure 2 TLC chromatograms of (1) methanolic extract, (2) hexane fraction, (3) DCM fraction and (4) butanol fraction of *C. hystrix* peel in reference to rutin, flavonoid standard (S) which were observed under a) 254 nm and b) 365 nm.

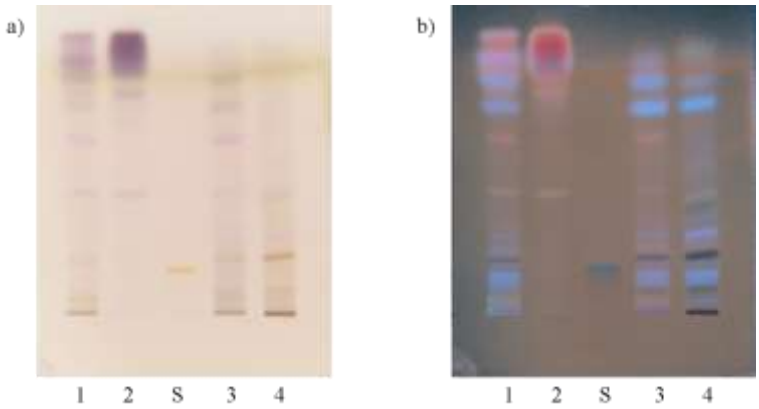

Figure 3 TLC chromatograms of (1) methanolic extract, (2) hexane fraction, (3) DCM fraction and (4) butanol fraction of *C. hystrix* peel in reference to the rutin, flavonoid standard (S) after being sprayed with anisaldehyde-sulfuric acid reagent and heated to 110ºC which were observed under a) white light and b) UV light at 365 nm

activity and to support further investigations on into the use of C. hystrix extract in the fight against GAS. Since molluscicidal activity of *C. hystrix* extract was found in the methanol, hexane and DCM fractions but not in the butanol fraction, the quantification of flavonoids, limonin and stability was not completed in the butanol fraction.

HPLC system suitability analysis was performed by testing HPLC conditions against reference standards established in previous studies.[26,27] Reproducibility of the system was obtained by repeating the experiment under the conditions described above. The retention times for limonin, naringin, hesperidin, and nobiletin were found to be the same as those reported in Chinapongtitiwat et al.[30]

The flavonoid and limonin (triterpene derivatives) content of the methanolic extract, hexane fraction and DCM fraction, were quantified by HPLC (Table 3 and 4). The highest amounts of flavonoid and limonin were found in the methanolic extract, followed by the DCM fraction and the hexane fraction respectively. The flavonoids found in the methanolic extract and DCM fraction were hesperidin, naringin

and nobiletin. However, the hexane fraction contained only hesperidin and naringin. The major flavonoid found in all *C. hystrix* peel extracts was hesperidin. Interestingly, it was noted that molluscicidal activities appeared to be in consistence with limonin and flavonoid contents in extract/fraction with higher activities. Furthermore, the flavoniod and limonin content found in our study were comparable with those found in crude methanolic extracts analyzed in previous reports.[30]

Stability studies of the methanolic extract, hexane, and DCM fractions were performed under static and accelerated conditions (Table 3-5). The amount of flavonoid and limonin found within the extract and fractions, before and after storage under accelerated conditions, are shown in Table 3-4.

In order to determine whether the flavonoid and limonin content would change significantly after storage in various conditions, which would affect anti-molluscicidal activities, the accelerating stability test was performed. After accelerated heating and cooling for 4 cycles, the flavonoids and limonin content did not change significantly. This suggests that the extracts were stable when kept under normal storage conditions. The physical characteristics of the extracts are reported in Table 5. The color remained brown-black after static and accelerated studies. The scent of the extracts was the scent of *C. hystrix* volatile oil. The scent decreased over time but still remained after the test. The extracts were dissolved in water to a concentration of 1 mg/mL for the pH measurements. It was found that the pH of the stored extracts was similar to that of the freshly prepared ones. Overall, the physical characteristics of all the extracts were unchanged after the stability studies.

Conclusion

In this work, *C. hystrix* peel was extracted in methanol and the crude extract was further isolated using organic solvents with various polarities, including hexane, DCM and butanol. Phytochemical screening and TLC chromatograms revealed various types of phytocompounds within the extracts including steroidal and triterpenoid saponin, triterpenes, phenolic compounds (flavonoids) and alkaloids. As confirmed by HPLC, the extracts of *C. hystrix* peel consisted of flavonoids such as hesperidin, naringin and nobiletin, including triterpenes represented by limonin. The highest level of compounds was found in the crude methanolic extract and the DCM fraction. The extract was stable under 25ºC storage where the physical and chemical

Table 3 Quantification of flavonoids in *C. hystrix* peel extracts

Extracts	Flavonoids content before storage (ppm)			Flavonoids content after storage (ppm)		
	Hesperidin	Naringin	Nobiletin	Hesperidin	Naringin	Nobiletin
Methanol	1225.0±144.0	123.0±26.9	83.0±12.6	1209.0±129.0	123.0±26.9	83.0±12.6
DCM fraction	925.0±104.2	93.0±19.6	44.9±13.6	909.7±109.0	88.0±12.9	43.2±9.9
Hexane fraction	125.0±14.2	14.7±9.9	Not detected	120.0±19.0	13.6±6.9	Not detected

Table 4 Quantification of limonin in *C. hystrix* peel extracts

Extracts	Limonin content before storage (ppm)	Limonin content after storage (ppm)
Methanol	215.0 ± 39.5	194.7 ± 32.2
DCM fraction	184.3 ± 34.0	175.3 ± 37.3
Hexane fraction	85.0 ± 13.9	74.7 ± 20.9

Table 5 Physical characteristics of *C. hystrix* peel extracts upon stability study

Extracts	Characteristics	Before storage	After storage		
			heating/cooling cycles	25 ºC with light (8 days)	25 ºC in the dark (8 days)
Methanol	Color	Brown-black	Brown-black	Brown-black	Brown-black
	Scent	*C. hystrix*	*C. hystrix*	*C. hystrix*	*C. hystrix*
	pH	7.57 ± 0.01	7.52 ± 0.02	7.54 ± 0.01	7.54 ± 0.00
Hexane fraction	Color	Brown-black	Brown-black	Brown-black	Brown-black
	Scent	*C. hystrix*	*C. hystrix*	*C. hystrix*	*C. hystrix*
	pH	7.48 ± 0.01	7.51 ± 0.00	7.53 ± 0.02	7.52 ± 0.01
DCM fraction	Color	Brown-black	Brown-black	Brown-black	Brown-black
	Scent	*C. hystrix*	*C. hystrix*	*C. hystrix*	*C. hystrix*
	pH	7.55 ± 0.01	7.47 ± 0.00	7.50 ± 0.01	7.52 ± 0.00

properties were not altered. Thus, our work illustrates that there are various types of phytocompounds contained within *C. hystrix* peel extract that can be isolated using different solvents and that these compounds could have a role in molluscicidal activity. Further compound identifications and quantification analysis should be performed in search of a potent phytocompound against the golden apple snail.

Acknowledgment

This work was supported by a project grant from Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand.

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