P-ISSN 2586-9000
E-ISSN 2586-9027
Homepage : https://tci-thaijo.org/index.php/SciTechAsia
Science & Technology Asia
Vol.24 No.1 January - March 2019
Page: [14-20]
Original research article

Extraction and Determination of Anthraquinone from Herbal Plant as Bird Repellent

Ruchira Khoomsab[1,*] and Kan Khoomsab[2]

[1]*Chemistry Program, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun 67000, Thailand.*
[2]*Education Science Program, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun 67000, Thailand.*

Received 24 September 2018; Received in revised form 29 December 2018
Accepted 11 January 2019; Available online 8 February 2019

ABSTRACT

The objective of this study was to compare the anthraquinone content of cassod tree leaves, golden shower pods and noni roots that were determined in the form of free anthraquinone and anthraquinone glycoside by Soxhlet and decoction extraction method. The anthraquinone compounds were identified by the Borntrager reaction. The determination of anthraquinone UV-Visible spectrophotometry at 325nm was used. The results show that the highest anthraquinone content in the golden shower pods with 70% (v/v) ethanol was 193.79mg/g dry plant. The suitable solvent for the anthraquinone glycoside extracts was 80% (v/v) ethanol for the cassod tree leaves. In the testing of birds, 1% of the extracted golden shower pods that cover the seeds for food consumption showed a lower amount than that consumed in the control group ($p < 0.05$).

Keywords: Extraction; Anthraquinone; Cassod Tree Leaves; Golden Shower Pods; Noni Roots.

1. Introduction

A chemical repellent is a substance to keep certain animals away from objects, areas, people, plants or other animals. Repellents generally work by taking advantage of an animal's natural aversion to something, and often what is chosen is something that the animal has learned to avoid (or instinctively avoid) in its natural environment [1]. Chemical repellents mimic natural substances that repel or deter animals or are designed to be so irritating to an animal or specific animal type that the target animal will avoid the object or area [2]. As a bird repellent, chemical repellents have been used to control birds in commercial and

***Corresponding author:** rupinkaew13@pcru.ac.th **doi:** 10.14456/scitechasia.2019.2

residential areas, in agricultural situations, and in airports.

Anthraquinone has recently been registered as a treatment to repel birds from turf and grass [3-4]. Ingestion of foods treated with anthraquinone can cause vomiting [4]. Presumably, the emetic response is produced by the irritation of the intestinal lining, but the actual mechanism is unknown. Anthraquinone is neither taste-repellent nor irritant on contact. Birds do not hesitate to eat treated foods and show no signs that the treated foods are unpleasant. Also, there was a research report that used this method in the agricultural field to prevent birds from damaging agricultural crops [5]. Kandel et al. [6] used Avidel, a brand of bird repellent spray with anthraquinone substance, to spray in a sunflower field to prevent birds from damaging the sunflower seeds. The study revealed that it was able to reduce the demolition of the sunflower seeds by the birds and receive greater yields than the control group. Similarly, Werner et al. [7] used the anthraquinone compound to prevent sunflower seeds from being demolished by blackbirds in the United States. In addition, the study by Avery et al. [8] discovered that the anthraquinone compound is also able to reduce the demolition of rice by red-winged blackbirds and boat tail grackles using 0. 5 and 1% (g/ g) of anthraquinone. Recent laboratory tests of an anthraquinone-based repellent revealed its repellent effectiveness as a seed treatment of agricultural crops for Canada geese, red-winged blackbirds and ring-necked pheasants [9-10].

Anthraquinones are commonly found as glycosides in living plants. They are found in the form of free anthraquinone and anthraquinone glycoside. The phytochemical substance of anthraquinone in plants is useful for its medicinal properties and as a food additive [11-12]. Anthraquinone is found in all parts of plants: roots, rhizomes, fruits, flowers, and leaves. Most of these compounds are derivatives of the basic structure 9,10-anthracenedione, a tricyclic aromatic organic compound with the formula $C_{14}H_8O_2$. [13-14]. In Thailand, there have been studies that investigated the amount of anthraquinone and compared the anthraquinone substance in roots, barks, stems, and leaves. [15-17]. Thailand is a research area of the literature related to anthraquinone extraction and analysis [11]. Therefore, the objective of this study was to extract and determine anthraquinone from local plants in Thailand, such as cassod leaves (*Senna siamea*), golden shower pods (*Cassia fistula*) and noni roots (*Morinda citrifolia*) as bird repellents. The comparison used different solvents in the extraction to obtain the total anthraquinone value and to analyze it using the UV-visible spectrophotometry technique.

2. Materials and Methods

2.1 Preparation of plant samples

Three species of plant samples, which are the noni roots, the golden shower pods and the leaves of the cassod tree, were taken from Phetchabun province. The plant samples were prepared by drying the sample of the plant at 110°C for 1h. Then, the samples of the plant were ground into powder and stored in the desiccator for further analysis.

2.2 Study of the extraction of Anthraquinone compounds in plants

Two extraction methods were used: boiling extraction and Soxhlet extraction with 70%, 80% and 95% ethanol solvent (v/v).

2.3 Boiling method

Boil 2g of ground dried plants in 200ml of water at 90 – 95°C for 7h. After that, evaporate and dry in the water bath, then weigh the sample and dilute it in the solvent for further analysis.

2.4 Soxhlet extraction method

Weigh a 2g sample of dry plant extracted using soxhlet with a solvent of 70%, 80% and 95% ethanol of 200ml using reflux 4 times and filter the extract with filter paper No. 1. Evaporate the samples to dryness in a water bath, then weigh the extracted sample and dilute in the solvent for further analysis.

2.5 Examination of Anthraquinone substance

The Bornträger-reaction was used to detect the anthraquinone substance aglycones in the extract by weighting 10g of added extract with a 1.0ml solution of 10% H_2SO_4, stirring, putting it in a hot water bath for 5 minutes, filtering insoluble parts and then letting the solution cool to room temperature. Remove the filtrate, and then add 0.5ml of 10% NH_3, shake it; if the substance is pinkish red, it indicates that there is anthraquinone substance.

2.6 Examination of Anthraquinone Glycoside substance

Weigh 10 grams of the extract and then add 15ml of 0.5N KOH. Add seven milliliters of 3% H_2O_2 and boil in the water bath, allow to cool and filter. Add ten drops of acetic acid, and then test it with pH paper. The sample solution was filtered and extracted with 15ml of chloroform. The extract was divided into two tubes, one for control and another for addition with NH_3 tube. Shake gently; a pinkish red color indicates anthraquinone glycoside.

2.7 Analyzing Anthraquinone substance

Ten milliliters of the sample was transferred to a 100 ml volumetric flask, adjusted for volume with solvent, and analyzed by UV-Vis spectrophotometer at a wavelength of 325 nm. The concentration was determined by checking the dilution factor of each sample compared to the standard anthraquinone solution.

2.8 Analyzing Anthraquinone Glycoside

The extraction protocol of Sakulpanich and Gritsanapan [16] was modified and used in this investigation. The UV-vis spectrophotometer was used to determine the anthraquinone glycoside in the sample by measuring the absorbance at 515nm. Each sample was made in triplicate.

2.9 Validation method for quqntitative analysis of anthraquinone by UV-Vis spectrophotometric method

Anthraquinone 98%, ACROS Organics™ was used as the standard for quantitative analysis of anthraquinone. The calibration curve of the standard was made from 5 concentrations. All concentrations were measured by UV-Vis spectrophotometric method at 325 nm. The measurement was done in triplicate. The relation between concentration and absorbance was plotted. The linearity was evaluated by regression analysis and residual of squares, and a correlation coefficient (r^2) was calculated.

2.10 Analysis of feeding preference experiments

Preparation of food for birds to feed pigeons. The grains were fermented with the extract of golden shower pods with a 1% (w/ v) concentration for 24 hours. The pigeons were chosen as test animals and captured in the area of Phetchabun province. The pigeons were individually caged (0.3 m long 0.3 m wide 0.3 m high) within an indoor aviary for at least two weeks prior to our preference experiments. Six pigeons were evaluated and three pigeons were used per treatment in the cages. The control group (0%) was fed with grains that are not coated with extracts of substances. The experimental group was fed with the grains covered with extractions of golden shower pods. We weighed 20 grams of food and fed the pigeons for 24 hours with bottles of water. We evaluated the food intake at the beginning and the weight after 24 hours. The analysis of the data was compared to know the differences between the average consumption of the control group and the experimental group with a statistical t-test.

3. Results and Discussion

3.1 Examination of Anthraquinone and Anthraquinone Glycoside substance

Borntrager's test is often used for its detection. It can be extracted with chloroform and separated by an aqueous solution (ammonia) [18]. The results found the substance in the three plant samples and when 10% NH_3 was added, the golden shower pods and the noni root appear to be pinkish red, while the leaves of the cassod tree were pale pink due to the obstruction of chlorophyll in the sample. Examination with anthraquinone glycoside from noni roots, golden shower pods, and cassod tree leaves, found that when NH_3 was added to the plant samples, red appeared in all the solvents, which indicated an anthraquinone glycoside substance in all reactions.

3.2 Quantitative Determination of Anthraquinone

The standard curve of the anthraquinone substance concentration when compared between 1-50mg/L and the absorbance at the wavelength of 325nm, the linear equation was $y = 0.0226x - 0.0418$ and $r^2 = 0.99$.

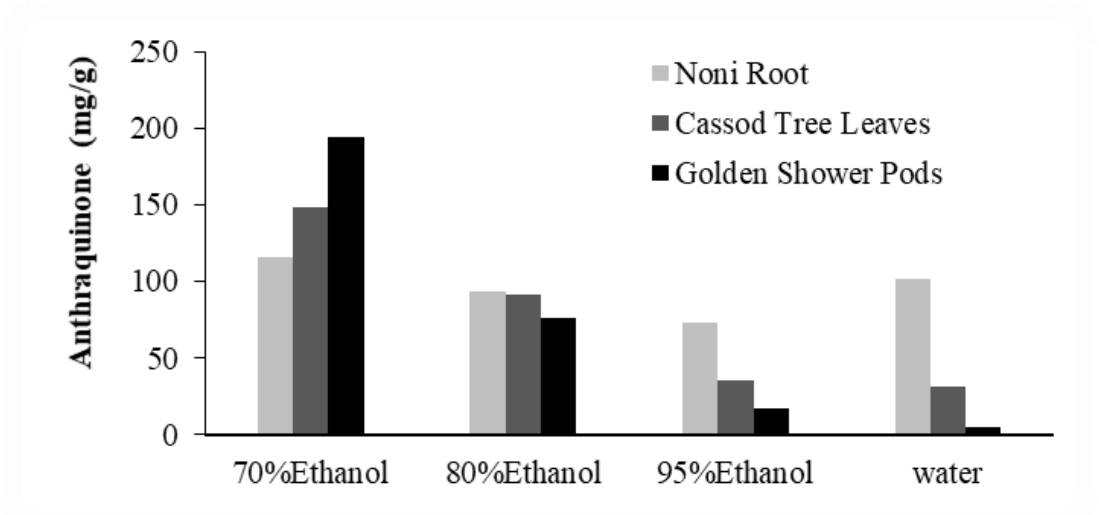

Fig. 1. The quantity of anthraquinone substance in noni roots, cassod tree leaves and golden shower pods.

The number of anthraquinones in the samples was compared with the anthraquinone standard (Fig. 1). The results show that the highest anthraquinone content of the golden shower pods with 70% (v/v) ethanol was 193.79 mg/g dry plant. Sakulpanich and Gritsanapan [16] reported maceration, percolation, Soxhlet extraction using 70% ethanol as solvent, producing more total anthraquinones than total anthraquinone glycosides.

3.3 Quantitative Determination of Anthraquinone Glycoside

In analysis of the quantity of anthraquinone glycoside from the reaction, the red solution is taken to measure the absorbance at 515 nm wavelength. The absorbance is shown in Fig. 2.

The absorbance analysis of the substance anthraquinone glycoside showed the highest absorbance value of 0.72 cassod tree leaves in 80% ethanol solvents. Sakulpanich and Gritsanapan [12] found

that the content of anthraquinone glycoside in the leaves and the decoction leaf extract of the golden shower (*C. fistula*) will be useful to find a good source of anthraquinone glycoside. Region and weather of cultivation are important factors affecting the amount of total anthraquinone glycosides in the leaves of *C. Fistula*. In the Central and North-East area of Thailand, the leaves contained a higher amount of total anthraquinone glycosides than the leaves samples collected from the South.

This study found that the appropriate solvent was 70% ethanol by Soxhlet extraction for anthraquinone extracted with golden shower pods and the appropriate solvent for anthraquinone glycoside extracts was 80% (v/v) ethanol by Soxhlet extraction with leaves of the cassod tree. The Soxhlet extraction was applied for the extraction of anthraquinone from dry parts of plants. Classical extraction techniques, such as maceration or Soxhlet extraction, are essentially based on the extraction capacity of different solvents and the application of heat and/or mixing. These methods are still in use as they provide a simple and inexpensive way to obtain essential oils and bioactive compounds from plant material. However, they are quite long: the Soxhlet extraction usually lasts from 4 to 48 h [11]. The Soxhlet extraction was applied for the extraction of anthraquinone from dried parts of plants and has been widely used since then. Due to its simplicity of use, low cost per sample and robust extraction device, this method is generally used as a reference. It is well established in official documents such as the US EPA, AOAC, and British standards because it offers very good performance [19].

3.4 Feeding consumption of grains coated with golden shower pod extraction by pigeons

We selected the golden shower pods to test as bird repellents because they offered the highest performance extraction. The result of this study found that the pigeons consumed less of the food with 1% of golden shower pods extracted than the amount consumed in the control group ($p < 0.05$) (Table 1).

For the golden shower pods extracted with the aversion behavior of pigeons, it is hypothesized that the substances classified in the anthraquinone group have an aversion effect to birds, due to the properties of anthraquinone which irritate the intestinal lining of the birds. If the bird consumes the food that affects its taste buds and makes its excretory system abnormal, then the bird will resist the continuous intake. Occasionally, ingestion of foods treated with anthraquinone causes vomiting, so the experience of eating foods treated with anthraquinone produces a conditioned aversion to that food [20]. However, this experiment was carried out using an extraction of Cassia tree seed pods that are supposed to have an anthraquinone compound. The results of this study found that it reflects resistance or refusal to eat foods consisting of the substance extracted from the golden shower pods. Suggestions for the next test of the experiment are to increase the concentration of the extract and also to experiment with other types of plants that contain anthraquinone components, such as noni, cassia siamese and the candle brush (*Senna tora*).

4. Conclusion

The use of chemical repellent to protect against avian feeding on agricultural crops is of great importance. Anthraquinone is a stable compound that is almost insoluble in water and of low toxicity to birds and mammals. The process of identifying and producing an effective and registered bird repellent chemical can be long, uncertain and expensive. The UV-Vis spectrophotometer is a fast, simple and

economical method to determine the concentration of an analyte in solution.

The anthraquinone content in cassod tree leaves (Senna siamea) , golden shower pods (*Cassia fistula*) and noni roots (*Morinda citrifolia*) are a good source of bird repellent substances. There are many species of Thai herbs that can repel birds and that contain chemicals to produce bird repellents.

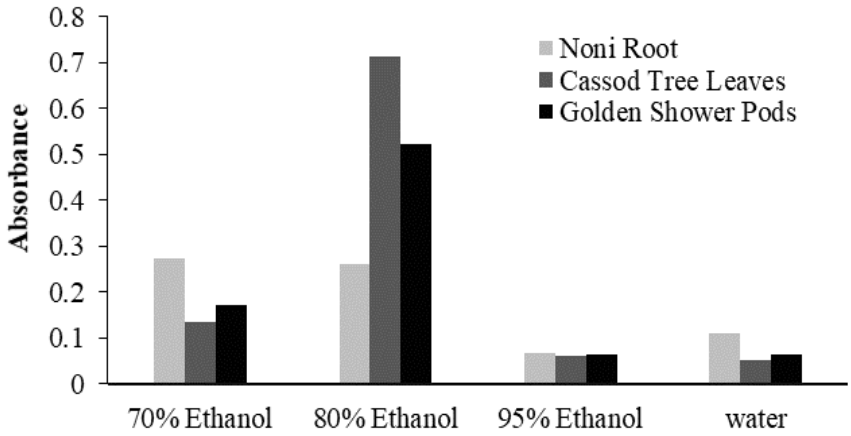

Fig. 2. The quantity of anthraquinone glycoside substance in noni roots, cassod tree leaves and golden shower pods.

Table 1. The average weight of bird food from the start and after 24 hours.

Group Experiment	Time		P - value
	0 hr.	**24 hr.**	
Control (g.)	20	3.45±0.26	8.43E-05
1% Extracted golden shower pods (g.)	20	11.13±0.77	

Acknowledgements

This study was financially supported by the National Research Council of Thailand and the Research and Development Institute, Phetchabun Rajabhat University.

References

[1] Clark L. Review of bird repellents. Proceeding of the eighteenth vertebrate pest Conference. 1998; 330-336.

[2] Ahmada S, Saleema Z, Jabeena F, Hussaina B, Sultanaa T, Sulta S. Potential of natural repellents methyl anthranilate and anthraquinone applied on maize seeds and seedlings against house sparrow (*Passerdomesticus*) in captivity. Braz J Biol. 2018;78(4):667–672.

[3] Blackwell B F, Seamans, TW, Dolbeer RA. Plant growth regulator (Stronghold™) enhances repellency of anthraquinone formulation (Flight-Control™) to Canada geese. J Wildl Manage.1999;63(4):1,336–1,343.

[4] Avery M L, Humphrey J S and Decker D G. Feeding deterrence of anthraquinone, anthracene, and anthrone to rice-eating birds. J Wildl Manage.1997; 61(4):1,359 -1,365.

[5] Eisemann J D, Werner S J and Jeanette R Z. Registration considerations for chemical bird repellents in fruit crops: Chemical bird repellents. Outlooks on Pest Management – April 2011. 2011;87– 91.

[6] Kandel H, Johnson, B, Deplazes, Linz, C

G, and Santer, M. Sunflower treated with Avipel (anthraquinone) bird repellent. USDA National Wildlife Research Center Staff Publications. 2009;928–937.

[7] Werner, S J, Tupper S K, Pettit S E, Ellis J W, Carlson, J C, Goldade D A, Hofmann N and M, Homan H J. Application strategies for an anthraquinonebased repellent to protect oilseed sunflo-wer crops from pest blackbirds. Crop production. 2014;59:63–70.

[8] Avery M L and Cummings J L. Chemical repellents for reducing crop damage by blackbirds. USDA National Wildlife Research Center-Staff Publications. 2003; 199:41-48.

[9] Avery M L, Humphrey J S, Primus T M, Decker D G and McGrane A P. Anthraquinone protects rice seed from birds. Crop Protection. 1998; 17(3):225–230.

[10] Werner S J, Carlson J C, Tupper S K, Santer, M M and Linz, G M. Threshold concentrations of and anthraquinone-based repellent for Canada geese, red-winged blackbird and ring-necked pheasants. Appl Anim Behav Sci. 2009;121(3-4):90–196.

[11] Duval J, Pecher V, Poujol M and Lesellier E. Research advances for the extraction, analysis and use of anthraquinone: A review. Industrial Crops and Product. 2016;94:812–833.

[12] Sakulpanich A and Gritsanapan W. Determination of Anthraquinone Glycoside Content in *Cassia fistula* Leaf Extracts for Alternative Source of Laxative Drug. Int J Biomed Pharmaceut Sci. 2009;3(1)42– 45.

[13] Ain J N, Sakinah A M M and Zularisam AW. Effects of Different Extraction Conditions on The Production of Anthraquinone. Aust. J.Basic & Appl. Sci. 2016;10(17):128–135.

[14] Bussmann R W, Hennig L, Giannis A. Ortwein J, Kutchan T M and Feng X. Anthraquinone Content in Noni (*Morinda citrifolia* L.) . Evid Based Complement Alternat Med. 2013;V. 2013,Article ID 208378,1–5.

[15] Temiyaputra W, Suebsanga T, Yajom K, Piyaworanon S and Leksawasdi N. Influences of Anthraquinone Extraction Techniques from *Morinda* sp. on Extraction Efficiency. Kasetsart J. (Nat.Sci.). 2008;42:118-126,

[16] Sakulpanich A and Gritsanapan W. Extraction Method for High Content of Anthraquinone fromk *Cassia fistula* Pods. J Health Res. 2008;22(4):167-172.

[17] Monton C, Duangjit S, Chankana N, Charoenchai L and Suksaeree J. The Optimization of the Anthraquinones Extraction from Senna tora Seeds Using Two Factor Spherical Composite Experimental Design. Proceeding of RSU International Research Conference 2017, Thailand, 2017;88–96.

[18] Yarnell E. Plant Chemistry in Veterinary Medicine: Medicinal Constituents and Their Mechanisms of Action. Veterinary Herbal Medicine; 2007;159–182.

[19] Genovese S, Tammaro F, Menghini L, Carlucci G, Epifano F and Locatelli M. Comparison of three different extraction methods and HPLC determination of the anthraquinones aloe-emodin, emodin, rhein, chrysophanol, and physcion in the bark of *Rhamnus alpinus* L. (Rhamnaceae). Phytochem. Anal. 2010; 21:261–267.

[20] Avery M L. Avian repellents. USDA National wildlife research center – staff publications. 2002; 122–129.