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Local plants as sources of the phytoecdysteroid, 20-hydroxyecdysone

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Abstract

Ecdysteroids derived their name from the insect molting process known as ecdysis. They are substances extensively explored as growth promoters for both plants and insects, essentially harmless to humans and appear to have a number of side effects that are considered to be beneficial. Physiologically active plant steroids known as phytoecdysteroids (PEs) contain structures like those of insect molting hormones are widely distributed in plants and acting as defense against phytophagous (plant-eating) insects. Numerous biological, pharmacological, and therapeutic features of PEs have been linked to their use in the treatment and prevention of both acute and chronic illnesses. The most abundant and common PE is the 20-hydroxyecdysone (20E). *Achyranthes aspera* L., *Amaranthus spinosus* L., *Ipomea pes-caprae* L. and *Portulaca oleracea* L are common local plants in the Philippines and were investigated to determine the presence of 20E through High Performance Liquid Chromatography. *Achyranthes aspera* L., *Amaranthus spinosus* L., and *Portulaca oleracea* L. contained 2 peaks close to the retention time of the target compound. *Ipomea pes-caprae* L., only one peak is observed within ± 0.120 minutes. Plant samples, *Ipomea pes-caprae* L. and *Portulaca oleracea* L. had peaks with retention times not significantly different from that of the standard were found, indicating presence of 20-hydroxyecdysone in these samples. The ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC/Q-TOF-MS) to confirm the presence or absence of 20E can be done as well to identify the other components of the samples, particularly those eluting near the retention time of 20E.

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Introduction

Ecdysis, from Ancient Greek “ékdusis” which means "stripping" or "the shedding of an exoskeleton in insects," is the root of the term of "Ecdysteroids" (ECs) (Arif *et al.*, 2022). The first EC, ecdysone, was isolated by Butenandt and Karlson from silkworm pupae as cited by Arif *et al.* (2022). Scientists began to demonstrate the roles for circulating hormones, like ecdysteroid in the development and maturation of insects in the early 20th century. It was initially noted in Stefan Kopec’s experiment that the insects’ brain, aside from its function in electrical signaling, is a secretory organ, controlling developmental processes through the release of a hormone into the bloodstream (Adams, 2003).

Cuticular growth and changes of most insects occur through molting. These processes are governed primarily by two classes of insect hormones- the molting hormones called ecdysteroids and juvenile hormone (JH) which regulates metamorphosis. Ecdysteroids are produced by the prothoracic glands of immature insects. In most insects, prothoracic glands degenerate, however, gonadal or other tissues produce ecdysteroids. Insects cannot synthesize steroids, consequently, sterols like cholesterol or closely related structure, are essential dietary constituents. In some cases, the conversion of cholesterol to the secreted ecdysteroid is accomplished by series of steps that involve several enzymes (P450). Other insects which may lack cholesterol from diets like phytophagous insects do their own precursor of ecdysteroids for their production (Chapman, 2013).

Phytoecdysteroids are a class of chemicals manufactured by plants for defense against phytophagous (plant eating) insects. These compounds are exact replicas of ecdysteroids, hormones used by the arthropod (insect) and crustacean (crab/lobster) families in the molting process known as ecdysis. (Al Naggar *et al.*, 2017).

There are two main hypothetical significance of phytoecdysteroids-they have hormonal role within the plant; second, they may participate in the defense of plants against non-adopted phytopagous invertebrates. They are widespread in more than 100 plant families covering the whole kingdom from ferns to angiosperms. Their contents in plants range from minute amounts to typically 0.1%, some organs may contain up to 3.2%, eg. in *Diploclistsia galucescenes* (Kreis and Muller-Uri, 2010).

The most common ecdysteroids in plants and animals are 20-hydroxyecdysone (20E) and polypodine B (polB) (Dinan and Lafont, 2006). Plants have a much greater diversity of ecdysteroid derivatives than arthropods, according to screening experiments on a variety of taxa. While insects primarily generate 20-hydroxyecdysone and alpha-ecdysone as physiological hormones, a single plant species often contains 20-hydroxyecdysone as the primary component and a complicated mixture of structurally related ecdysteroids in minimal amounts. The first plant-derived ecdysteroids, known as ponasterones A, B, and C, were identified in the conifer *Podocarpus nakaii* Hayata in 1966. Other ecdysteroids, including 20-hydroxyecdysone, were discovered in *Podocarpus elatus* R.Br., a fern *Polypodium vulgare* L., and not long after *Hypnum fauriei* and *Achyranthes faurieri* Lev. Vaniot, an angiosperm from the Amaranthaceae family. These early discoveries in taxonomically diverse species piqued researchers' interest, and it was soon apparent during a screening study that the presence of ecdysteroids was widespread in the plant kingdom. These secondary metabolites are abundant in a number of taxa. Ecdysteroids were discovered in more than half of the fern species examined, which were from the Polypodiaceae, Pteridaceae, and Blechneaceae families. Ecdysteroids can also be found in a variety of conifers and angiosperm species from the Caryophyllaceae, Amaranthaceae, Chenopodiaceae, Asteraceae, and Lamiaceae families. The genus Silene is particularly noteworthy, as it contains a huge number of different ecdysteroids, Thiem, B. *et al.* (2017).

PEs have significant impact on health and disease which included biological, pharmacological, and medicinal properties. They appear to be non-toxic to

animals and have a variety of advantageous pharmacological effects (adaptogenic, anabolic, anti-diabetic, hepatoprotective, immunoprotective, wound-healing, and perhaps even anti-tumour) (Dinan, 2009).

Niranjan Das, *et al.* (2021) also emphasized that PEs have antioxidant properties that regarded as natural substances to prevent and postpone oxidative stress and collagenase-related skin damage. PEs have demonstrated anabolic action in a number of *in vitro* and *in vivo* investigations, with a typical increase in growth and skeletal muscle mass as well as an increase in fiber size through muscle-specific effects in various animals. The blood glucose-lowering abilities of PEs due to their direct stimulation of pancreatic -cells raise the possibility of their usage in diabetic conditions. PEs have shown potent growth-inhibitory activity, hence, a promising anticancer compound.

This study explored on characterizing identified local plants which are potential sources of PEs and High Performance Liquid Chromatography (HPLC) was utilized to further investigate the presence of the specific PE, 20-hydroxyecdysone (20E). It employed laboratory experimental research which eventually compared the chromatograms of the standard, 20E and sample plants as to their retention time and peak areas.

Materials and methods

The identified plants, *Achyranthes aspera* L., *Amaranthus spinosus* L, *Ipomoea pes-caprae* L., *Portulaca oleceracea* L., properly described by De Guzman-Ladion (1985), Kurian (2010) and Stuart (n.d.), were collected along agronomic, horticultural fields and idle lands in the locality. The plant parts, leaves and stems were used except that of I. pes-caprae L. that utilized the seeds. The leaves and stems were properly washed with tap water and rinsed with distilled water, and air-dried until plant parts become well dried for grinding. After drying, the plant materials were mechanically ground into fine powder.

Preparation for Rotary Evaporation

For every 50 g of the powdered plant material, 150mL of the solvent (methanol) were used and soaked for 72 hours. After which, these were filtered using Whatman filter paper No. 4. The filtrate was subjected to extraction through rotary evaporation following the 20/40/60 rule, the condenser temperature was 20°C, vapor was 40°C, bath temperature was 60°C, and 74.51 torr for the pressure.

Preparation of Samples for HPLC

Approximately 100mg of the slurry portion of each of the samples were weighed and transferred into 2mL microcentrifuge tubes. This was done in triplicates.

After weighing, 1mL of 50:50 H2O:MeOH was added to the sample ten capped immediately. This was then subjected to sonication at 25°C for 5 min then centrifuged at 14,000 rpm for 5 min. The supernatant was obtained using the 1000µL micropipette then transferred into 2mL amber vials for analysis.

High Performance Liquid Chromatography (HPLC)

The presence of the phytoecdysteroids, specifically the 20-hydroxyecdysone was confirmed through High Performance Liquid Chromatography (HPLC) analysis outsourced from the De La Salle University, Central Instrumentation Facility, Chromatography Laboratory, Biñan, Laguna, Philippines. The specific standard, 20-Hydroxyecdysone (C27H44O7) powder, ≥93% (HPLC) from Sigma Aldrich was purchased through a local distributor.

The HPLC made use of Agilent 1260 Infinity II HPLC using the detector G1314B Variable Wavelength Detector, InfinityLabPoroshell 120 EC-C18 4.6 x 150mm with 4micron particle size column, LCMS-grade acetonitrile (w/0.1% formic acid) and deionized water (w/0.1% formic acid) for the mobile phase, LCMS-grade methanol (MeOH) and deionized water for the reconstitution, 2mL microcentrifuge tubes, 1000µL micropipette, sonicator bath, microcentrifuge, and 2mL amber vials with silicon/PTFE septa screwcaps. The column oven temperature (°C) was at 40 during the test.

Standard Preparation

Exactly 0.1mg of the standard was weighed and transferred into a 2mL microcentrifuge tube then

1mL of 50:50 H2O:MeOH was added, the microcentrifuge was capped, sonicated at 25°C for 5 min then centrifuged at 14,000 rpm for 5 min. This was transferred into a 2mL amber vial for analysis.

HPLC Conditions

The injection volume was 5µL; the flow rate was at 1.000mL/min, and the Mobile phase: Gradient of 0.1% Formic Acid in Water and 0.1% Formic Acid in Acetonitrile; the Max column pressure was at 400 bar; column oven temperature was at 40°C; the variable wavelength detector was 242 nm; the run time was 23 min; and equilibration time was 7 min.

Data Analysis

Chemstation software was used for data acquisition and chromatogram processing and the Welch's t-test for significant difference between retention time of sample peaks versus standard peak was used.

Results and discussion

The standard 20-hydroxyecdysone was analyzed prior to the HPLC analyses of the sample. Figure 2 shows the chromatogram for 100mg/L (ppm) of 20-hydroxyecdysone prior to plant samples analysis while Figure 3 presents the chromatogram for 100mg/L (ppm) of 20-hydroxyecdysone after plant samples analysis, in addition, Table 1 shows the average retention time and peak area of the standard, 20-hydroxyecdysone.

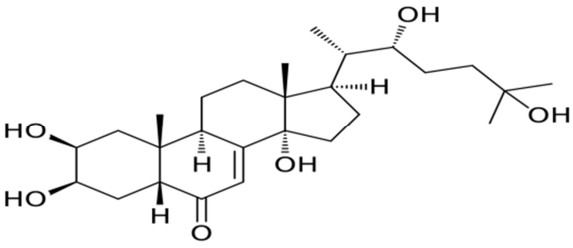

Fig. 1. Chemical structure of the 20-hydroxyecdysone.

Characteristics and chromatograms of the plant samples

Achyranthes aspera or prickly chaff is an herbaceous plant species in the family Amaranthaceae which nurtures in open fields and waste lands to a height of about one meter with elliptic or round leaves. It is easily identified through its 50 cm long spikes upon maturity, bearing numerous very tiny greenish-white flowers which stand upright. The spines with the seeds easily stick on to clothing which is a good method of seed dispersal (J C Kurian, 2010).

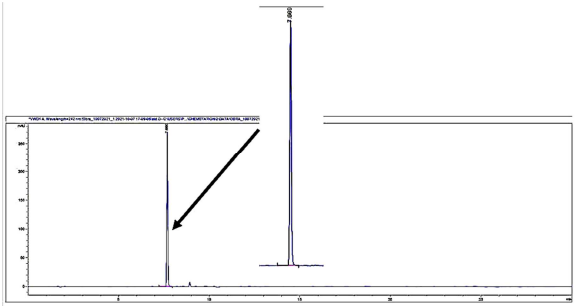

Fig. 2. Chromatogram of 100 ppm 20-hydroxyecdysone, analyzed prior to samples (Chromatogram A).

Table 1. Average retention time and peak area in the standard chromatograms.

Retention time (min)	Peak area (min*mAu)
7.670 ± 0.001	1038.60 ± 0.57

It is also characterized as erect or ascending herbs or shrubs; 0.8-4 m high, sometimes almost treelike. Stems are tough, that turns into a woody stem at its base. Leaves are opposite, simple and ovate, up to 10 cm long by 8 cm wide, which tapers to a point at both ends and shortly stalked, the leaf blades are entire. Inflorescences are both terminal and axillary with only a few flowers open at the same time. The flowers are hermaphrodite, solitary in axils of acute, membranous with persistent bracts, individual flowers are small, with five white to pink or greenish tepals and white filaments, and form narrow, elongated terminal spikes up to 60 cm long. As the flowers age, they bend downwards and become pressed closely against the stem. The bracts surrounding the flowers in the fruiting stage have sharp, pointed tips making the heads spiny to the touch. The fruits are capsules, orange to reddish purple or brown, 1-3 (-5) mm long with 1-seeded ovary (Rojas-Sandoval and Acevedo-Rodríguez, 2012).

The chromatogram of *Achyranthes aspera* is shown in Figure 4 while Table 2 illustrates the average retention time and peak area of the peaks with the

retention time close to the standard's retention time by a ± 0.120 deviation. Test for significant difference of the peak retention time versus the retention time of the standard's peak was determined using Welch's t-test. Retention times for both peaks were found to be significantly different from that of the standard (p values < 0.01), indicating possibility that both peaks are not 20-hydroxyecdysone, but compounds belong to the same class. Nevertheless, the peak with an average retention time of 7.688, closer to the retention time of the standard, has higher likelihood of belonging to 20-hydroxyecydysone.

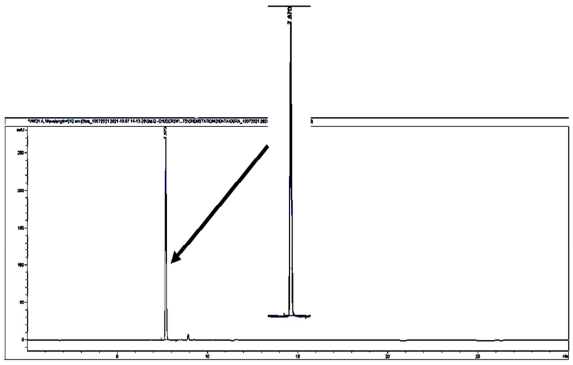

Fig. 3. Chromatogram of 100 ppm 20-hydroxyecdysone, analyzed after the plant samples.

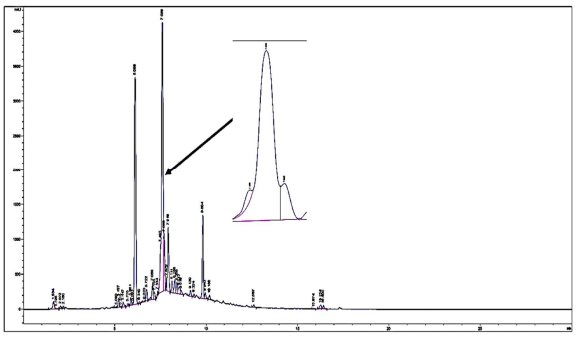

Fig. 4. Chromatogram of *A. aspera*.

Table 2. Average retention time and peak area of the target compound in *A. aspera*.

Peak No.	Retention time (min)	Peak Area (min*mAu)
1	7.586 ± 0.004**	22103.73 ± 1264.29
2	7.688 ± 0.005*	2834.50 ± 310.87

Notes: Welch's test for significant difference between retention time of sample peaks versus standard peak: *p < 0.05; **p < 0.01; ***p < 0.001. Confidence interval = 95%; df = 2

Amaranthus spinosus Linn. from the Amaranthaceae family is an annual or perennial herb, believed to originate from low and tropical south and Central America and was introduced into warmer parts of the world. This weed is widely distributed worldwide including the United States of America, and all tropical and subtropical regions of Africa, Southeast Asia and India (S. Asha *et al.*, 2016).

It is known as spiny amaranth which is stout, erect, smooth, branched-herb, 0.4 to 1 meter high. Stems are armed with slender, axillary spines. The presence of spines differentiates it from "kolitis" (*Amaranthus viridis*). Leaves are glabrous, long-petioled, oblong to oblong ovate, or elliptic-lanceolate, 4 to 10 centimeters long, obtuse and alternate. It has very numerous flowers, stalkless, green, about 1 millimeter long, and borne in dense, axillary clusters and in elongated terminal axillary spikes. The sepals are 5 or 1-3, ovate to linear, and often aristate. The petals are scarious; bracts are linear, bristle—pointed and as long as the sepals or longer. The fruits are utricles, wrinkled, nearly as long as the sepals. The seeds are minute, black and shining. It is found throughout the Philippines at lowlands and low altitudes, in open waste place, gregarious and abundant along sand bars and margins of streams. It was probably introduced and now, pantropic (Stuart, n.d.).

Sable KV and Saswade RR (2017) revealed the phytochemical analysis of various extracts of leaves yielded alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenol, amino acids and proteins, saponins, tannins, terpenoids, quinones and resins.

The chromatogram of *A. spinosus* is shown in Figure 5 and Table 3 displays the average retention time and peak area of the peak with the retention time close to the standard's retention time by a ±0.120 minutes deviation. Test for significant difference of the peak retention time versus the retention time of the standard's peak was determined using Welch's t-test. Retention times for both peaks were found to be significantly different from that of the standard (p-values < 0.01), indicating the possibility that both

peaks were not 20-hydroxyecdysone, but compounds belonging to the same class. Nevertheless, the peak with an average retention time of 7.679, closer to the retention time of the standard, had higher likelihood of belonging to 20-hydroxyecydysone.

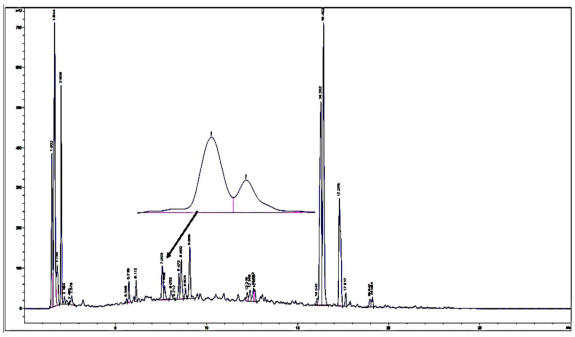

Fig. 5. Chromatogram of *A. spinosus*.

Table 3. Average retention time and peak area of the target compound in *A. spinosus*.

Peak No.	Retention time (min)	Peak Area (min*mAu)
1	7.554 ± 0.003***	367.30 ± 88.26
2	7.679 ± 0.001**	114.50 ± 38.07

Notes: Welch's t-test for significant difference between retention time of sample peaks versus standard peak: *p < 0.05; **p < 0.01; ***p < 0.001; Confidence interval = 95%; df = 2

Ipomoea pes-caprae or beach morning glory from the family Convolvulaceae is a wide-spreading, creeping or twining, smooth vine. Leaves are alternate, orbicular to elliptic, thick, shining, 6 to 14 cm long, with a notched or lobed tip and broad base. Flowers are campanulate, light purple, borne on pedicels in the axils of leaves, usually as long as the stalks. Stalk is erect and bears one to six flowers, which often opens one at a time. Sepals are green, elliptic, and 8 mm long. Corolla is purple, bell-shaped, and 5 cm long, with the limb 5 to 6 cm in diameter and slightly lobed. Capsules are smooth, ovoid, about 1 cm long. Seeds are covered with hairs (Stuart, n.d.). Extracts of the plant were investigated for the presence of major class of compounds like alkaloids, flavonoids, saponins, tannins and glycoside. Phytochemicals yielded the presence of steroids, terpenoids, alkaloids and flavonoids (Ganjir *et al.*, 2013) while the seeds contain most abundant oleic acid (Knothe, 2017).

The chromatogram of *I. pes-caprae* is shown in Figure 6 and Table 4 displays the average retention time and peak area of the peak with the retention time close to the standard's retention time by a ±0.120 minutes deviation. Test for significant difference of the peak retention time versus the retention time of the standard's peak was determined using Welch's t-test. Retention time for the peak was found to be not significantly different from that of the standard, indicating that the peak was likely to be 20-hydroxyecdysone.

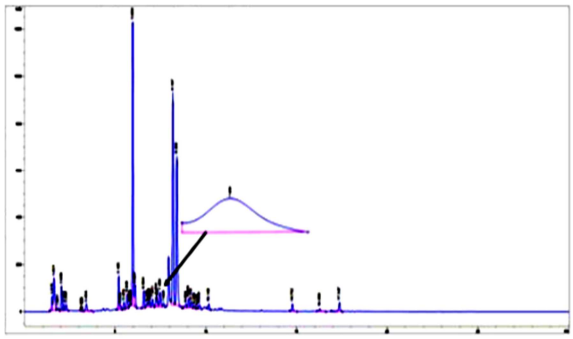

Fig. 6. Chromatogram of *I. pes-caprae*.

Table 4. Average retention time and peak area of the target compound in *I. pes-caprae*.

Peak No.	Retention time (min)	Peak Area (min*mAu)
1	7.668 ± 0.003	122.93 ± 63.43

Note: Welch's test for significant difference between retention time of sample peaks versus standard peak: *p < 0.05; **p < 0.01; ***p < 0.001; Confidence interval = 95%; df = 2

The findings of Ganjir, *et al.* (2013) which explored on the leaves of *I. pes-caprae* found out that the extract confirmed the presence of steroids, terpenoids, alkaloids, and flavonoids which were phytoecdysteroids but not necessarily 20-hydroxyecdysone.

Portulaca oleracea L. is a warm-climate, herbaceous succulent annual plant with a cosmopolitan distribution belonging to the Portulacaceae family. It is commonly known as purslane (USA and Australia), rigla (Egypt), pigweed (England), pourpier (France), and Ma-Chi-Xian (China). It is an annual, prostrate or spreading, succulent, branched, smooth, often

purplish herb, with the stems 10 to 50 cm long. Nodes are without appendages. Leaves are fleshy, flat, oblong-obovate, 1 to 2.5 cm long, with obtuse apex and wedge-shaped base. Flowers are yellow, stalkless, axillary and terminal few-flowered heads. Heads are solitary or cymose with compressed buds. Petals are five and yellow, about as long as the sepals and notched at the tip. Flowers open only for a few hours in the morning. Fruits are capsules, which come out horizontally containing many minute, dark brown, heart-shaped seeds. It is distributed widely in the tropical and subtropical areas of the world including many parts of the United States and is eaten extensively as a potherb and is added to soups and salads around the Mediterranean and tropical Asian countries. *P. oleracea* also provides a source of nutritional benefits owing to its rich omega-3 fatty acids and antioxidant properties. The constituents of *P. oleracea* have been isolated including flavonoids, alkaloids, fatty acids, terpenoids, polysaccharides, vitamins, sterols, proteins, and minerals. The flavonoid contents of *P. olecacea* vary according to the parts of the plant on which the roots contain the highest levels followed by the stem and the leaf. There are seven different flavonoids present in the plant that includes kaempferol, myricetin, luteolin, apigenin, quercetin, genistein, and genistin. Moreover, *P. oleracea* contains monoterpenes such as portulosides A and B, diterpenes such as portulenes, and β-amyrin type triterpenoids (Yan-Xi Zhou, 2015).

Study of methanolic extract of *P. oleracea* yielded up to 0.532mg (in 100g of dry powder) of ecdysterone in addition to ƒsitosterol. Ecdysterone is a powerful natural anabolic steroid which increases growth of muscle protein, blood cells and stamina, and reduces fat, without any known side effects. Ecdysterone has been found to have a number of medicinal properties viz., antidiabetic, cardioprotective, anti-inflammatory and hepatoprotective (Daniel and Mammen, 2014).

The chromatogram of *P. oleracea* is shown in Figure 7 and Table 5 shows the average retention time and peak area with the retention time close to the standard's retention time by a ±0.120 minutes deviation. Test for significant difference of the peak retention time versus the retention time of the standard's peak was determined using Welch's t-test. Retention time for Peak 1 was found to be significantly different from that of the standard, indicating possibility that the peak was not 20-hydroxyecdysone, but compounds belonging to the same class. The retention time of Peak 2 on the other hand, was found to be not significantly different from that of the standard, indicating that the peak was likely to be 20-hydroxyecdysone.

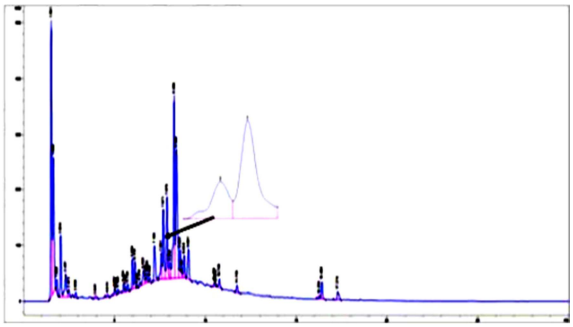

Fig. 7. Chromatogram of *P. oleracea*.

Table 5. Average retention time and peak area of the target compound in *P. oleracea*.

Peak No.	Retention time (min)	Peak Area
1	7.560 ± 0.003***	235.13 ± 28.54
2	7.670 ± 0.002	593.63 ± 39.90

Note: Welch's test for significant difference between retention time of sample peaks versus standard peak: *p < 0.05; **p < 0.01; ***p < 0.001; Confidence interval = 95%; df = 2

P. oleracea contained two (2) peaks close to the retention time of the target compound while there was only one (1) peak observed within ± 0.120 minutes in *I. pes-caprae*. It was found out further with the use of Welch's t-test (standard vs plant source of PE) that the peaks with retention time in *I. pes-caprae* and *P. oleracea* were not significantly different from that of the standard which indicated the presence of 20-hydroxyecdysone in these plant samples.

The above results confirmed that of Yan-Xi Zhou *et al.* (2015) that included the presence of phytoecdysteroids in *P. oleracea* namely - monoterpenes such as portulosides A and B, diterpenes such as portulenes,

and β-amyrin type triterpenoids and that of Daniel & Mammen (2014) confirming the presence of ecdysterone in addition to ƒsitosterol.

Conclusion

Achyranthes aspera L., *Amaranthus spinosus* L., and *Portulaca oleracea* L. contained 2 peaks close to the retention time of the target compound. *Ipomea pes-caprae* L., only one peak is observed within ± 0.120 minutes. Plant samples, *Ipomea pes-caprae* L. and *Portulaca oleracea* L. had peaks with retention times not significantly different from that of the standard were found, indicating presence of 20-hydroxyecdysone in these samples. The aforementioned local plants are therefore potential sources of phytoecdysteroids specifically, 20-hydroxyecdysone.

Recommendations

Given that the retention times of the peaks in plant samples *A. aspera*, and *A. spinosus,* and Peak 1 of plant sample, *P. oleracea* are significantly different from the retention time of 20-hdroxyecdysone in Chromatograms A and B, it is recommended to analyze the samples using UHPLC-QTOF to confirm the presence or absence of 20-hydroxyecdysone in the samples, as well as to identify the other components of the samples, particularly those eluting near the retention time of 20- hydroxyecdysone. There are a lot of local plants in the Philippines needed to be explored and these are potential sources of phytoecdysteroids. Further investigations shall be done to identify the specific phytoecdysteroids present in them.

References

Adams Michael. 2003. Ecdysteroids. Encyclopedia of Insects. Resh, V & Carde, R. Eds. Academic Press, Elsevier Science, USA.

Arif Y, Singh P, Bajguz A, Hayat S. 2022. Phytoecdysteroids: Distribution, Structural Diversity, Biosynthesis, Activity, and Crosstalk with Phytohormones. Int J Mol Sci **23(15),** 8664. DOI: 10.3390/ijms23158664. PMID: 35955797; PMCID

Asha S, Rekha R, Sadiq AM. 2016. *Amaranthus spinosus*- A review. Bulletin of Environment, Pharmacology, and Life Sciences **5(9).** Acadey for Environment and Life Sciences, India. eISSN: 2277-1808.

Chapman RF. 2013. The Insects. Structure and Function, 5th ed., Cambridge University Press, UK.

Daniel M, Mammen D. 2014. "Ecdysterone and Antioxidants in Purslane (Portulaca oleracea Linn.)." Asian Journal of Chemical and Pharmaceutical Research, AJCPR **2(2),** 137-139.

Dinan L, Lafont R. 2006. Effects and applications of arthropod steroid hormones (ecdysteroids) in mammals. The Journal of endocrinology **191(1),** *1-8.*

Dinan Laurence. 2009. The Karlson Lecture. Phytoecdysteroids: what use are they?. Archives of insect biochemistry and physiology **72(3),** 126-141. https://doi.org/10.1002/arch.20334

Ganjir Minakshi, Dipti Ranjan Behera, Sunita Bhatnagar. 2013. "Phytochemical anlysis, csytotic and antioxidant potential of Ipomoea pes-caprae (L) R. Br and *Merrenia umbellata* (L) H. Hallier". International Journal of Scientific & Technology Research **2(5).**

Knothe G, Luis F Razon, Domingo A Madulid, Esperanza Maribel G Agoo, Maria Ellenita G de Castro. 2017. "Fatty Fatty Acid Profiles of Garuga floribunda, Ipomoe apes-caprae, Melanolepis multiglandulosa and Premna odorata Seed Oils." Journal of the American Oil Chemists' Society **94(2).**

Kreis W, Muller-Uri F. 2010. "Biochemistry of sterols, cardiac glycosides, brassinosteroids, phytoecdysteroids and steroid saponins". Biochemistry of Plant Secondary Metabolism. Annual Plant Reviews **Vol. 40,** 2nd ed., Wink, M., Ed. John Wiley & Sons, Ltd., Publication, 304-347

Kurian JC. 2010. Amazing Healing Plants **Vol. 1.** Philippine Publishing House, ISBN 978-971-581-211-5.

Ladion-De Guzman H. 1985. Healing Wonders of Herbs. Philippine Publishing House, Manila, Philippines. ISBN 971-1019-01-9.

Niranjan Das, Siddhartha Kumar Mishra, Anusha Bishayee, Euns S Ali, Anupam Bishayee. 2021. The phytochemical, biological, and medicinal attributes of phytoecdysteroids: An updated review, Acta Pharmaceutica Sinica B.

Rojas-Sandoval and Acevedo-Rodríguez. 2012. Achyranthes aspera (Devil's horsewhip). CABI Compendium. https://doi.org/10.1079/cabicom pend ium.2664

Sable KV, Saswade RR. 2017. Preliminary phytochemical analysis of *Amaranthus spinosus* leaves; International J. of Life Sciences **5(4),** 742-745.

Stuart, G. n.d. "Bagasua" retrieved from http:// www. stuartxchange.com/Bagasua.html

Stuart, G. n.d. "Kolitis" retrieved from http:// www. stuartxchange.com/Kolitis.html

Thiem B, Kikowska M, Maliński MP, Dariusz Kruszka, Marta Napierała and Ewa Florek. 2017. Ecdysteroids: production in plant in vitro cultures. Phytochem Rev **16,** 603-622. https://doi.org/10.1007/s11101-016-9483-z

Yahya Al Naggar, Mohamed Ghorab, Kariman Mohamed. 2017. "Phytoecdysteroids: Isolation and Biological Applications." American Journal of Life Sciences **5(1),** pp. 7-10. doi: 10.11648/j.ajls. 20170

Yan-Xi Zhou, Hai-Liang Xin, Khalid Rahman, Su-Juan Wang, Cheng Peng, Hong Zhang. 2015. "Portulaca oleracea L.: A Review of Phytochemistry and Pharmacological Effects." Review Article. BioMed Research International. Vol 2015, Article ID 925631, Hindawi Publishing Corporation.