**Pharmacogn.** Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

# Regulation of Pro-Inflammatory Enzymes by the Dragon Fruits from *Hylocereus undatus* (Haworth) and Squalene - its Major Volatile Constituents

**Ibrahim M. S. Eldeen[1,2], Shin Y. Foong[3], Noraznawati Ismail[1], Keng C. Wong[3]**

[1]Institute of Marine Biotechnology, University Malaysia Terengganu, Kuala Terengganu, Malaysia, [2]Department of Silviculture, Faculty of Forestry, University of Khartoum, Khartoum, Sudan, [3]School of Chemical Sciences, University Sains Malaysia, Minden, Penang, Malaysia

Submitted: 25-06-2019 Revised: 22-08-2019 Published: 31-03-2020

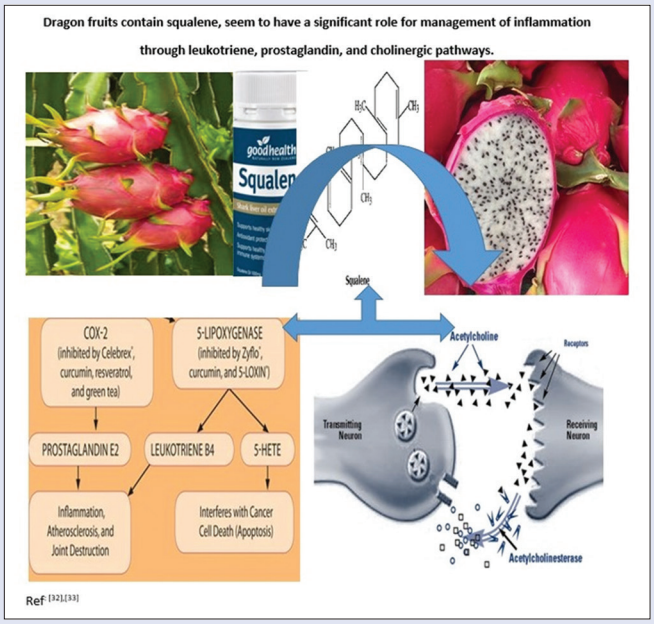
## ABSTRACT

**Background:** *Hylocereus undatus* produces fruits known as dragon fruit (Pitaya). The fruit and other parts of *Hylocereus* species are used for the treatment of diuretic, gastritis, kidney problems, and wound healing. **Objectives:** To investigate anti-inflammatory properties of the flesh and peel of Pitaya and to identify the major bioactive constituent(s). **Materials and Methods:** The flesh and the peel of the fruit were blended separately with water, vacuum-distilled, freeze-dried, and then used for the bioassay tests against the 5-Lipoxygenase (5-Lipox), Cyclooxygenase-2 (COX-2), and Acetylcholinesterase (AChE) enzymes. **Results:** Squalene appeared to be the dominant constituent (13.2%) in the flesh of the fruit. The crude flesh extract and the squalene showed strong activities against AChE with inhibition percentage >80% ($IC_{50}$ <43 μg/mL). The peel was active against 5-Lipox with inhibition of 81%. Inhibition percentages recorded for the positive controls used were 77% for Zileuton against 5-Lipox ($IC_{50}$ 28 μg/mL), 85% for the Galantamine against AChE ($IC_{50}$ 16 μg/mL), and 86% for the Celecoxib against COX-2 ($IC_{50}$ 32 μg/mL). For maximum efficacy against the three enzymes, squalene showed the best performance with $EC_{50}$ values ranging between 46 and 47 μg/mL. **Conclusion:** The findings showed that Pitaya has a significant potential for the management of inflammatory conditions through different mechanisms including, leukotriene, prostaglandins, and cholinergic pathways. To the best of our knowledge, identification of squalene in the dragon fruit flesh and the validated biological activities were not reported previously and therefore accounted for the novelty of the study.

**Key words:** Anti-inflammatory, cholinergic, cyclooxygenase, dragon fruit, leukotriene, nutraceuticals

## SUMMARY

- Pitaya has a significant potential for the management of inflammatory conditions through different mechanisms including, leukotriene, prostaglandins, and cholinergic pathways. The study also confirmed the presence of squalene in the flesh of the dragon fruit *H.undatus*. This indicates the health promoting properties of the dragon fruit as a nutraceutical diet. These properties in addition to the chemical contents known for the peel as a source of phenolic compounds will lead to various applications in food related industry and health.




**Abbreviations used:** ATCI: Acetylcholine iodide; AChE: Acetylcholinesterase enzyme; DTNB: 5,5-dithiobis-2-nitrobenzoic acid; BSA: Bovine serum albumin; HFLS: Human fibroblast like synoviocytes; GC-MS: Gas chromatography-mass spectrometry; COX-2: Cyclooxygenase-2; 5-Lipox: 5-Lipoxygenase; MTT: Abbreviation for the dye compound 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

**Correspondence:**

Dr. Ibrahim M. S. Eldeen,
Institute of Marine Biotechnology,
University Malaysia Terengganu,
21030 Kuala Terengganu, Malaysia.
E-mail: eldeen24@gmail.com
**DOI:** 10.4103/pm.pm_271_19

**Access this article online**
**Website:** www.phcog.com
**Quick Response Code:**

## INTRODUCTION

*Hylocereus undatus* is a fruit plant indigenous to tropical and subtropical America. It produces fruits known as dragon fruit and Pitaya. The fruit comes in a number of varieties including Red Pitaya from *H. undatus* (red skin-white flesh) and *Hylocereus polyrhizus* (red skin-red flesh), and Yellow Pitaya (yellow skin-white flesh) form *Hylocereus megalanthus*.[1]

Pitaya received worldwide recognition especially in Asian countries such as Vietnam, Taiwan, Malaysia, and the Philippines, as an ornamental plant for its large, scented, night-blooming flowers and multipurpose



**Cite this article as:** Eldeen IM, Foong SY, Ismail N, Wong KC. Regulation of pro-inflammatory enzymes by the dragon fruits from *Hylocereus undatus* (Haworth) and squalene - its major volatile constituents. Phcog Mag 2020;16:S81-6.

uses which enable it to be of high economic potential.[2] Pitaya fruit juice contains wide range of chemical constituents such as citric and ascorbic acids, proteins, riboflavin, niacin, and several minerals such as Ca, P, Fe, in addition to phenolic compounds including gallic acid and catechins.[3] The seeds of the fruits of *Hylocereus* fruits were also reported to be rich in polyunsaturated fatty acids and linoleic acid.[4] However, no detailed data made available on the volatile constituents of *Hylocereus* fruits flesh.

Different parts of *Hylocereus* species were reported to be used for medicinal purposes. The leaves and flowers of *H. undatus* were used by the old Mayas for hypoglycemic purposes, as a diuretic and healing agent.[5] The fruits and flowers used for gastritis and kidney problems.[6] Perez *et al*.,[7] studied the wound healing properties of aqueous extracts from different parts of *H. undatus* including fruit pulp. Based on their observation, they stated that a topical application of *H. undatus* caused a remarkable increase in hydroxyproline, tensile strength, and improved epithelialization. Effects of extracts from some cacti were also linked with the central nervous system (CNS) stimulants and regulators of blood pressure, sleep, hunger, and thirst.[8] Pitaya seeds were also reported to contain oil with high level of functional lipids in addition to their biological properties.[1,4,9]

Phytochemical analysis of the peel of *Hylocereus* species indicated that the peel is rich of betalains which comprises the red-violet betacyanins and yellow betaxanthins. Betacyanins are known to possess some biological properties with potential application in pharmaceutical industry. Luo *et al*.,[10] reported results of a phytochemical analysis and *in vitro* evaluation of extracts of pitaya (dragon fruit) peels from *H. undatus* and *H. polyrhizus,* in which the main components of *H. undatus* were found to be β-amyrin, γ-sitosterol, and octadecane. According to the authors, these compounds were responsible for the observed cytotoxic activities of the extracts. This was similar to a previously reported antiproliferative activity of red Pitaya peel in melanoma cells.[11]

Due to the popularity and wide usage of the dragon fruits and the reputable medicinal properties, we attempt to investigate the potential anti-inflammatory properties of extracts from the flesh and peel of *H. undatus.* This report highlights phytochemical analysis, and bio-efficacy of extracts from the dragon fruit of *H. undatus* against the pro-inflammatory enzymes 5-Lipoxygenase (5-Lipox), Cyclooxygenase-2 (COX-2), and Acetylcholinesterase (AChE). The report also highlights inhibitory effects of squalene, as a major constituent identified in the flesh extract, against the three enzymes.

## MATERIALS AND METHODS

The name *H. undatus* (Haw.) Britton and Rose has been checked with http://www.theplantlist.org/tpl1.1/record/kew-2856879 (accessed on May 2018). This name is the accepted name of this species in the genus *Hylocereus* (data supplied on March 23, 2012). The plant materials were purchased from a local fruit hypermarket in Kuala Terengganu, Malaysia (GPS coordination: 5o22'52.93"N, 103o04'57.58"E). The sample was authenticated by Dr. Suliman Eldeen, a curator in the institute and a voucher specimen (Eldeen 39-D) was deposited. All the fruits were first washed with distilled water and the peel removed. The flesh (300 g) and the peel (180 g) were blended separately with 300 mL water and then immediately vacuum-distilled for 5 h, yielding approximately 220 mL and 150 mL of distillate from the flesh and peel respectively. Only the flesh distillate was used for phytochemical analysis by extraction with dichloromethane (5 mL × 25 mL). The extract was then concentrated using a Kuderna-Danish concentrator at a bath temperature 50°C and reduced to a final volume of 0.1 mL under a gentle stream of $N_2$ gas at room temperature before gas chromatography (GC) analysis. The vacuum distillation was performed in duplicate by adding heptadecane (2.4 mg) and dotriacontane (2.3 mg) to the macerated fruits to estimate the efficiency of the vacuum distillation.

### Gas chromatography-mass spectrometry

The GC–flame ionization detection (GC–FID) analysis was carried out using a Thermo Finnigan instrument (San Jose, CA, USA). Briefly, two fused-silica capillary columns SPB-1 and Supelcowax 10 were used. The initial oven temperature for both the columns was set at 40°C for 5 min, then to 220°C at 5°C/min and held for 15 min. Injector port and detector temperatures were set at 250°C. The percentages of the peak area of each components [Table 1] were the average values of two injections of the isolated volatiles. The absolute amounts of the major components were calculated from the peak area percentages using the predetermined response factors, and the mean values from the two injections. The retention indices (RI) relative to those of n alkanes (C5-C-32) were calculated.

With the implementation of the same capillary GC conditions as described above, the GC/mass spectrometry (MS) analysis was performed using a Perkin-Elmer Clarus 600T (Waltham, MA, USA). The compounds were identified by the comparison of their mass spectra with the data of authentic compounds or with reference spectra recorded in the library. The findings were further analyzed against the RI obtained with those of authentic compounds or with data in the literature.[12]

### Biological tests and *in vitro* bioassay models used

Fifty milliliter of each of the distillated flesh and peel were subjected to a water evaporator and concentrated to dryness. The concentrated solution was then freeze-dried to yield 11 and 7 mg for flesh and peel, respectively. The dried materials were used for the bioassay tests. Squalene appeared to be the major constituents as indicated by the GC-MS analysis. Thus, a pure squalene compound (≥98%) liquid with molecular weight of 410.72, CAS Number: 111-02-4, was purchased from Sigma Aldrich (Catalogue no. S3626) and subjected for the biological tests.

### 5-Lipoxygenase inhibitor screening assay

The biological performances of the extracts and the squalene against the 5-Lipox enzyme inhibitory effects were evaluated using the 5-Lipox inhibitor screening assay kit (Item No. 760700; Cayman Chemical, USA). A detailed description about the method is available in our previous reports.[13,14] The test materials were dissolved in methanol to the concentration of 100 µg/mL, while the positive control used (Zileuton) was dissolved to a concentration of 50 µg/mL. The arachidonic acid (10 µL) was added to each well of the 96-well plate used to initiate the reaction. The plate was placed on a shaker for 5 min after which the chromogen was added to stop the reaction. The Graph pad prism was used to calculate the $IC_{50}$ values, and the results presented are the means of three separate tests.

### Cyclooxygenase inhibitor screening assay

The inhibition of prostaglandin biosynthesis by the extracts and the squalene was assessed using the COX inhibitor screening assay kit (No. 560131; Cayman Chemical, USA) based on the manufacturer's protocol provided with the kit. The method of the test was fully described in our earlier reports.[13,14] The drug celecoxib was used as a positive control.

### Acetylcholinesterase enzyme inhibitory activity

The assessment of the inhibitory effects on AChE biosynthesis by the extracts, and squalene was carried out using the microplate assays. A detailed description of the bioassay can be found in our previous reports.[13-15]

# RESULTS

## Gas chromatography-mass spectrometry analysis and identification of the major constituents

On low-temperature vacuum distillation, the Pitaya fruit from *H. undatus* afforded essences which possessed the aroma characteristic of the fruits. The identified volatile constituents, together with the GC-FID peaks areas, are presented in Table 1. Fifty-nine compounds (constituting 99.5% of the sample by peak area per cent) were identified. A total of 23 alcohols were detected accounted for about 37% of the volatiles, of which 1-hexanol, 1-octadecanol, 1-hexadecanol, and 1-tetradecanol were clearly dominant. Carboxylic acids were the second most abundant constituents with the linoleic acid, oleic acid, and palmitic acid being the main derivatives. From an aroma standpoint, aldehydes are important flavor contributors. Eight aldehydes, accounting for 1.2% of the isolate, were detected. Hexanal, (E)-2-hexenal, nonanal, decanal, (E, E)-2, 4-decadienal together with hexanol and the unsaturated C6 alcohols, (E)-3-hexen-1-ol, (Z)-3-hexen-1-ol and (E)-2-hexen-1-ol, presumably contributed to the fatty-green odor of the fruit. Squalene appeared to be the most dominant constituents in the Pitaya flesh with a high proportion of 13.2% of the peak area. Squalene is a polyunsaturated hydrocarbon with a formula of $C_{30}H_{50}$ and formed by six isoprene units. It has gained attraction after being identified in the shark liver extract and therefore considered as the main source of the compound in addition to some other natural sources such as olive oil.[16,17] The occurrence of squalene at such high concentration in the flesh of the dragon fruit in this study was noteworthy, and to the best of our knowledge, this triterpene has not been reported as a fruit volatile in the flesh of *H. undatus*. However, squalene was reported presence in a lower concentration in the peel extracts of both *H. undatus* and *H. polyrhizus*.[10]

**Table 1:** The volatile constituents identified in the flesh of Pitaya from *Hylocereus undatus*

| Compound | Retention indices | | Area (%)[a] | Concentration (mg/kg)[b] |
|---|---|---|---|---|
| | Supelcowax 10 | SPB-1 | | |
| Hexanal | 1079 | 762 | 1.4±0.06 | - |
| Ethylbenzene | 1123 | 841 | - | - |
| (E)-2-hexenal | 1222 | 831 | 6.2±0.04 | - |
| Styrene | 1257 | | 0.9±0.01 | - |
| Tridecane | 1300 | 1300 | 9.1±0.01 | - |
| 1-Hexanol | 1357 | 873 | 21.1±0.99 | 2.1 |
| (Z)-3-hexen-1-ol | 1384 | 855 | 0.6±0.03 | - |
| (E)-2-hexen-1-ol | 1405 | | 2.9±0.14 | - |
| 1-Tetradecanol | 2172 | 1670 | 2.5±0.08 | - |
| octadecanal | 2300 | 2002 | 0.6±0.05 | - |
| (6Z, 10Z)-Geranyl linalool | 2351 | 2012 | 1.3±0.04 | - |
| 1-Hexadecanol | 2376 | 1870 | 7.1±0.13 | 0.6 |
| 1-Octadecanol | 2585 | 2076 | 2.7±0.08 | 0.2 |
| Tetradecanoic acid | 2710 | 1765 | 0.5±0,02 | - |
| Geranylgeraniol | 2731 | 2089 | 1.3±0.06 | - |
| pentadecanoic acid | 2790 | 1869 | 1.0±0.14 | - |
| Octacosane | 2800 | | 0.5±0.03 | - |
| Palmitic acid | 2892 | 1965 | 9.6±0.52 | 0.1 |
| Palmitoleic acid | 2905 | 1826 | 8.2±0.45 | - |
| 2-Monolinolein | | 2436 | 0.5±0.02 | - |
| Squalene | 3023 | 2540 | 13.2±0.08 | 0.9 |
| Octadecanoic acid | 3107 | 2161 | 1.1±0.1 | - |
| Oleic acid | 3143 | 2137 | - | - |
| Linoleic acid | | 2130 | 1.3±0.08 | - |
| stearic acid | | 2157 | 1.1± | - |
| Total constituents with peak area (%) ≥0.5 | | | 92.31 | |
| Total | | | 99.5 | |

The mass spectrum and retention indices of each of the listed constituents agreed with the relevant reference compounds. Constituents with peak area <0.5% are not listed. [a]Total FID area obtained (%) on Supelcowax 10 capillary column, calculated as the mean value of two injections, [b]The major components calculated from the peak area (%) using the relative response factors of the FID for the compounds. FID: Flame ionization detection; -: Not detected

**Table 2:** Inhibition of 5-lipoxygenase, cyclooxygenase-2 and Acetylcholinesterase enzymes by the dragon fruit flesh and peel extract, and squalene compound as detected using the *in vitro* biological models

| Isolates and positive controls tested | Biological activities obtained as inhibition (%) and $IC_{50}$ values (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | 5-lipoxygenase | | Cyclooxygenase -2 | | Acetylcholinesterase | |
| | Inhibition (%) | $IC_{50}$ | Inhibition (%) | $IC_{50}$ | Inhibition (%) | $IC_{50}$ |
| Dragon flesh extract | 60±3.1 | 73±2.2 | 66±2.1 | 76±1.5 | 87±1.8 | 35.6±1.7 |
| Dragon peel extract | 81±3.1 | 71±2.4 | 57±3.5 | 78±0.7 | 69±2.3 | 51±1.3 |
| Squalene | 69±2.4 | 64±2.4 | 69t±1.8 | 71±2.3 | 83±2.6 | 42±2.8 |
| Zileuton | 77±1.4 | 28±2.7 | - | - | - | - |
| Celecoxib | - | - | 86±0.8 | 32±0.4 | - | - |
| Galanthamine | | | | | 85±2.2 | 16±0.3 |

Inhibition (%) was obtained at a concentration of 100 (µg/mL) for the crude extract and squalene. Inhibition (%) of the standard drugs, celecoxib, zileuton and galanthamine were obtained at a concentration of 50 (µg/mL). IC: Inhibitory concentration

## Biological activities observed by the extracts and the squalene compound

Inhibition of the pro-inflammatory enzymes 5-Lipox, COX-2, and AChE enzymes by the dragon fruit flesh and peel extracts, squalene, and the positive standard drugs (Zileuton, Celecoxib, and Galanthamine) were obtained using the bioassay models as described earlier. The obtained results are presented in Table 2. The dragon flesh extract possessed high inhibitory effects against AChE with inhibition percentage of 87% and $IC_{50}$ value of 35 µg/mL. The extract, however, showed moderate performance against both COX-2 and 5-Lipox. Interestingly, similar effects were also observed by the squalene compound against AChE, and with slightly higher activities against COX-2 and 5-Lipox. Extract of the peel appeared more active against 5-Lipox with inhibition of 81% but was found weaker against AChE and COX-2. Inhibition percentages recorded for the positive controls used were 77% for Zileuton against 5-Lipox ($IC_{50}$ 28 µg/mL), 85% for the Galanthamine against AChE ($IC_{50}$ 16 µg/mL), and 86% for the Celecoxib against COX-2 ($IC_{50}$ 32 µg/mL).

The concentration responses of the tested materials and the standard drugs against the three enzymes were assessed using the relevant *in vitro* bioassay models for each test as mentioned earlier. Five different concentrations (25, 50, 75, 100, and 150 µg/mL) were used in three replicates [Figure 1]. Results were analyzed using graph pad prism to obtain the response curves and the maximum efficacy ($E_{max}$) for each sample.

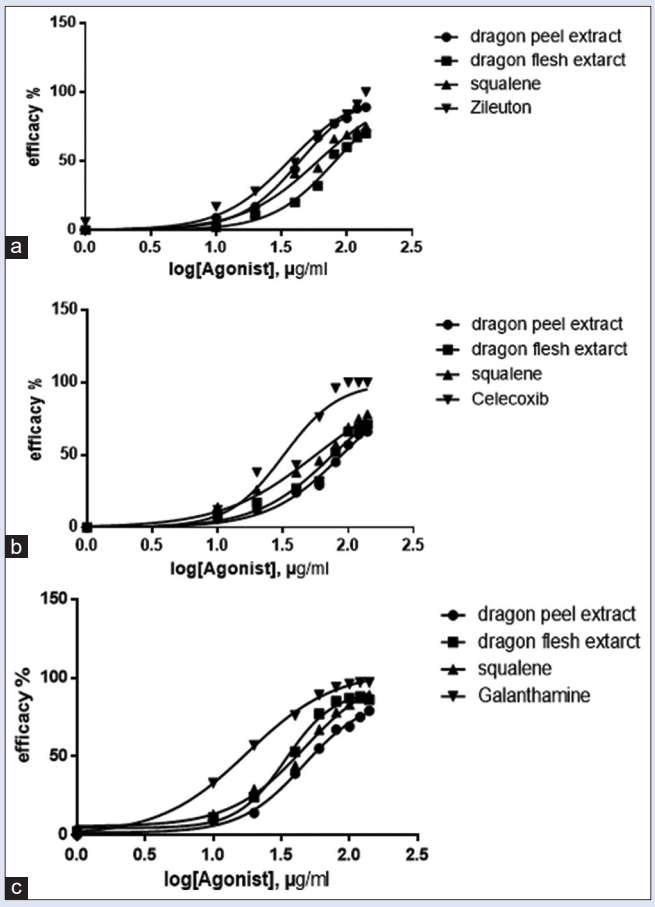



**Figure 1:** Concentration response of the flesh, peel extracts of the dragon fruit, the squalene and the standard drugs (positive controls) against: (a) 5-Lipoxygenase enzyme; (b) Cyclooxygenase enzyme; and (c) Acetylcholinesterase enzyme

The $E_{max}$ (%) obtained against 5-Lipox by the tested samples at a concentration of 150 µg/mL were 89 (peel extract), 70 (flesh extract), 75 (squalene), and 100 (zileuton). $EC_{50}$ obtained were 43, 69, 47, and 41 for the peel, flesh, squalene, and zileuton, respectively.

$E_{max}$ obtained by the peel, flesh, and squalene at a concentration of 150 µg/mL against COX-2 were 66% ($EC_{50}$ = 74), 71% ($EC_{50}$ = 58), and 78% ($EC_{50}$ = 46), respectively. $E_{max}$ of 100% was recorded for the Celecoxib at a concentration of 100 µg/mL with an $EC_{50}$ value of 11 µg/mL.

For AChE enzymes, the flesh extract and squalene showed relatively similar performance when tested at concentration of 100 µg/mL with an $E_{max}$ of 87% (flesh extract, $EC_{50}$ = 34 µg/mL) and 83% (squalene, $EC_{50}$ = 46 µg/mL). The peel extract, however, at this concentration showed $E_{max}$ of 69% with an $EC_{50}$ of 46 µg/mL. $E_{max}$ observed for the galanthamine at concentration of 100 µg/mL was 96% with an $EC_{50}$ value of 17 µg/mL [Figure 1]. The flesh of the dragon fruit in this study showed the best performance against AChE followed by the peel against 5-Lipox. As per assessment of the overall performance against the three enzymes, squalene appeared to be the best performer with $EC_{50}$ values ranging between 46 and 47 µg/mL. This was followed by the peel extract against 5-Lipox and AChE.

## DISCUSSION

In this study, we investigated phytochemical analysis and anti-inflammatory properties of the dragon fruit (Pitaya) from *H. undatus* against three pro-inflammatory enzymes using *in vitro* models to reveal part of the possible underlying mechanism of actions. Both Lipox and COX pathways are found to be involved in the inflammatory actions. Discovery of molecules that inhibit both enzymes (COX-2 and 5-Lipox) would be an advantageous by targeting both proteins.[13] In addition to this, there are other mechanisms that contribute to the regulation of inflammatory response through the immunomodulatory circuit termed the cholinergic anti-inflammatory pathway. These mechanisms indicating the role of acetylcholine in macrophage deactivation and the involvement of the vagus and cholinergic receptors in the process.[18] Although it is argued that acetylcholine necessary for the cholinergic anti-inflammatory pathway are synthesized by T lymphocytes and not neural in origin, suppression of AChE enzyme will decrease the rapid hydrolysis of acetylcholine leading to enhancement of vagal stimulation and inhibition of localized pro-inflammatory factors.[19,20] Extract from the flesh of dragon fruit in this study showed remarkable activities against the three enzymes, with stronger inhibition toward acetylcholinesterase enzyme. This indicates that the fruit can play a role on alleviation of inflammatory symptoms through mechanism related to cholinergic anti-inflammatory pathway. Moreover, the effects on both Lipox and COX enzymes observed in the flesh extract are interesting as it indicates its high potentiality for dual COX/Lipox effects which lead to blockage of both prostaglandins and Leukotrienes pathways. This indication of anti-inflammatory properties observed here is in agreement with a number of previous reports highlighted the medicinal properties of dragon fruits.[21,22] Perez *et al.*[7] reported anti-inflammatory effects of aqueous extracts of different parts of *H. undatus* including rind and fruit pulp. They concluded that topical applications of *H. undatus* can increase the production of hydroxyproline, tensile strength, and total proteins and therefore facilitate the healing process.

Squalene was appeared to be the major constituents of the dragon fruit flesh; therefore, it may be responsible of the activities observed by the flesh extract in this study. This assumption is supported by the activities recorded for the squalene compound against the three enzymes tested which possessed relatively similar performance to that recorded for the flesh extract.

Squalene is a natural lipid belonging to the terpenoid family. Various classes of plant terpenoids have found to be effective as anti-inflammatory agents. Anti-inflammatory properties of squalene were previously indicated through different mechanisms including its role as an antioxidant agent which can protect the skin surfaces from lipid peroxidation.[23] It was also reported to ameliorate atherosclerotic lesions through the reduction of CD36 scavenger receptor expression in macrophages.[24-26] Cárdeno *et al.*[22] investigated effects of squalene against pro-inflammatory mediators and pathways modulating overactivation of neutrophils, monocytes, and macrophages. The authors revealed that squalene downregulated gene expression of COX-2 among other genes in lipopolysaccharide-activated human neutrophils and monocytes. They concluded that squalene has a significant role for the management of inflammatory conditions. These findings are in agreement with our results obtained for squalene against COX-2 enzyme. Moreover, our results showed high effect of squalene against AChE enzyme. This may suggest that the compound also regulates inflammation through the cholinergic pathway.[13-15] The remarkable inhibition of AChE activity by both squalene and the flesh extract in this study indicated their potential for therapeutic uses in the treatment of cognitive functions with relation to CNS disorders. Aguilera *et al.*[16] studied effects of squalene on a fetal alcohol syndrome, a disorders of the CNS associated with growth retardation. The authors concluded that squalene can be used to prevent the neuronal and glial damages. These findings support our current results obtained for squalene compound against AChE enzyme as one of the key players in CNS actions and inflammation process.

Dragon fruit peels are reported to contain higher concentration of betalains and can therefore be used as a source for pigment extraction. Betalains are plant colorants composed of a nitrogenous core structure betalamic acid. They are responsible for the cyanic colors of flower petals and fruits. Several studies confirmed the high radical scavenging activity of betalains.[27,28] The peel of the dragon fruit in this study possessed higher activity against 5-Lipox enzyme followed by acetylcholinesterase but was weaker against COX-2. This may suggest that the anti-inflammatory effects of the peel act through mechanism other than prostaglandin pathway. However, inhibition of COX enzyme by betalains was previously suggested as one of the mechanisms for its anti-inflammatory properties.[29] The low inhibitory effect against COX-2 observed in this study may be due to the low concentration of the betalains in the extract used. Variation in chemical composition and structure of betalains from different sources may also lead to a different biological hits. Nonetheless, Rodriguez *et al.*[30] investigated anti-inflammatory properties of dragon fruit peel and revealed that its health-promoting properties attributed to its betalain content. Red beetroot betalains were also found to exert strong anti-inflammatory activity on carrageenan-induced paw edema with underlying mechanisms believed to be through inhibition of pro-inflammatory cytokines tumor necrosis factor-alpha and interleukin.[31] These findings support our current results indicating anti-inflammatory properties of the peel of the dragon fruits. The activities observed here may be due to the presence of betalain in the extract, with similar scenario for underlying mechanism of actions.

## CONCLUSION

Our results in this study confirmed the presence of squalene in the flesh of the dragon fruit from *H. undatus* and showed the potential anti-inflammatory properties of the flesh and the squalene. These findings indicate the health promoting properties of the dragon fruit as a nutraceutical diet to prevent some of neuronal and inflammatory related disorders. To the best of our knowledge, identification of squalene among the volatile constituents in the dragon fruit flesh and the assessed biological functions observed in this study were not reported elsewhere and therefore indicating the novelty of this work. The biological activities recorded for the dragon fruit peel are noteworthy as the peel is a waste material from consumption of the fruit. The obtained results suggest that the peel has significant potential for management of inflammatory-related conditions characterized by regulation of 5-Lipox and AChE enzymes. These properties in addition to the chemical contents known for the peel as a source of phenolic compounds will lead to various sophisticated applications in food-related industry and health. The obtained results represent a reference point for further research on the role of dragon fruit flesh and peel extracts on diseases associated with prolong inflammatory conditions. Currently, we are focusing on the effects of the extracts on rheumatoid arthritis using 3-dimensional pannus model.

## Financial support and sponsorship

This work was supported by the Fundamental Research Grants (FRGS grant vot: 59321) from the Ministry of Higher Education, Malaysia, and the Research Grant RU1001/PKIMIA/811050 offered by University Sains Malaysia (USM)-Penang, Malaysia.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Lim HK, Tan CP, Karim R, Ariffin AZ, Bakar J. Chemical composition and DSC thermal properties of two species of *Hylocereus* cacti seed oil*: Hylocereus undatus* and *Hylocereus polyrhizus*. Food Chem 2010;119:1326-31.
2. Jeronimo MC, Orsine JC, Carvalho MR. Nutritional pharmacological and toxicological characteristics of pitaya (*Hylocereus undatus*): A review of the literature. Afr J Pharm Pharmacol 2017;11:300-4.
3. Lim TK. Edible Medicinal and Non-Medicinal Plants. Fruits. Vol. 1. Heidelberge, London, New York: Springer Dordrechin; 2012. p. 636-55.
4. Arrifin A, Bakar J, Tan CP, Rahman RA, Karim R, Loi CC. Essential fatty acids of pitaya (dragon fruit) seed oil. Food Chem 2009;114:561-4.
5. Arguete AV, Cano LM, Rodarte ME. Atlas of the Plants of Mexican Indigenous Medicine. Pharmacognosy Res 1994;2:31-5.
6. Donadio CD, Nachtgal JC, Sacramento CK. Exotic Fruits (Frutas Exóticas). São Paulo, Brazil: Funep Jaboticabal; 1998. p. 279.
7. Perez G RM, Vargas S R, Ortiz HY. Wound healing properties of *Hylocereus undatus* on diabetic rats. Phytother Res 2005;19:665-8.
8. Franco-Molina M, Gomez-Flores R, Tamez-Guerra P, Tamez-Guerra R, Castillo-Leon L, Rodríguez-Padilla C. *In vitro* immunopotentiating properties and tumour cell toxicity induced by *Lophophora williamsii* (peyote) cactus methanolic extract. Phytother Res 2003;17:1076-81.
9. Phebe D, Chew MK, Suraini AA, Lai OM, Janna OA. Red-fleshed pitaya (*Hylocereus polyrhizus*) fruit colour and betacyanin content depend on maturity. Int Food Res J 2009;16:233-42.
10. Luo H, Cai Y, Peng Z, Liu T, Yang S. Chemical composition and *in vitro* evaluation of the cytotoxic and antioxidant activities of supercritical carbon dioxide extracts of pitaya (dragon fruit) peel. Chem Cent J 2014;8:1-7.
11. Wu LC, Hsu HW, Chen YC, Chiu CC, Lin YI, Ho JA. Antioxidant and antiproliferative activities of red pitaya. Food Chem 2006;2:319-27.
12. Marques FD, McElfresh JS, Millar JG. Kovats retention indexes of monounsaturated C12, C14, and C16 alcohols, Acetates and aldehydes commonly found in lepidopteran pheromone blends. J Braz Chem Soc 2000;11:592-9.
13. Eldeen IM, Ringe J, Ismail N. Inhibition of pro-inflammatory enzymes and growth of an induced Rheumatoid Arthritis Synovial Fibroblast by *Bruguiera cylindrica*. Int J Pharmacol 2019;15:916-25. [Doi: 10:3923/ijp].
14. Eldeen IM, Mohamed H, Tan WN, Siong JY, Andriani Y, Tengku-Muhammad TS. Cyclooxygenase, 5-lipoxygenase and acetylcholinesterase inhibitory effects of fractions containing, a-guaiene and oil isolated from the root of Xylocarpus moluccensis. Res J Med Plants 2016;4:286-94.
15. Eldeen IM, Abdul H, Wong KC, Abdullah H, Tengku-Muhammad MA, Abdillahi HS, *et al. In*

*vitro* repression of cyclooxygenase, acetylcholinesterase activities and bacterial growth by trans-phytol and a glycolipid from the leaves of *Homalomena sagittifolia*. Res J Med Plants 2016;10:320-9.

16. Aguilera Y, Dorado ME, Prada FA, Martínez JJ, Quesada A, Ruiz-Gutiérrez V. The protective role of squalene in alcohol damage in the chick embryo retina. Exp Eye Res 2005;80:535-43.
17. Kim SK, Karadeniz F. Biological importance and applications of squalene and squalane. Adv Food Nutr Res 2012;65:223-33.
18. Pavlov VA, Ochani M, Gallowitsch-Puerta M, Ochani K, Huston JM, Czura CJ, *et al*. Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. Proc Natl Acad Sci U S A 2006;103:5219-23.
19. Jänig W, Green PG. Acute inflammation in the joint: Its control by the sympathetic nervous system and by neuroendocrine systems. Auton Neurosci 2014;182:42-54.
20. Martelli D, McKinley MJ, McAllen RM. The cholinergic anti-inflammatory pathway: A critical review. Auton Neurosci 2014;182:65-9.
21. Reddy LH, Couvreur P. Squalene: A natural triterpene for use in disease management and therapy. Adv Drug Deliv Rev 2009;61:1412-26.
22. Cárdeno A, Aparicio-Soto M, Montserrat-de la Paz M, Bermudez B, Muriana FJ. Squalene targets pro- and anti-inflammatory mediators and pathways to modulate over-activation of neutrophils, monocytes and macrophages. J Funct Foods 2015;14:779-90.
23. Kabuto H, Yamanushi TT, Janjua N, Takayama F, Mankura M. Effects of squalene/squalane on dopamine levels, antioxidant enzyme activity, and fatty acid composition in the striatum of Parkinson's disease mouse model. J Oleo Sci 2013;62:21-8.
24. Granados-Principal S, Quiles JL, Ramirez-Tortosa CL, Ochoa-Herrera J, Perez-Lopez P, Pulido-Moran M, *et al*. Squalene ameliorates atherosclerotic lesions through the reduction of CD36 scavenger receptor expression in macrophages. Mol Nutr Food Res 2012;56:733-40.
25. Soehnlein O, Lindbom L. Phagocyte partnership during the onset and resolution of inflammation. Nat Rev Immunol 2010;10:427-39.
26. Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. Nat Rev Immunol 2011;11:762-74.
27. De Mello FR, Bernardo C, Dias CO, Gonzaga L, Amante ER, Fett R, *et al*. Antioxidant properties, quantification and stability of betalains from pitaya (*Hylocereus undatus*) peel. Cienc Rural 2015;45:323-8.
28. Belhadj Slimen I, Najar T, Abderrabba M. Chemical and antioxidant properties of betalains. J Agric Food Chem 2017;65:675-89.
29. Nunes S, Serra A, Bronze M, Simplicio A, Matias A, Duarte C. Bioactive extracts derived from fruits (*Prunus avium* and *Opuntia ficus-indica*) as potential natural anti-inflammatory modulators in inflammatory bowel diseases. Eur J Pharmacol 2011;668:S1-38.
30. Rodriguez EB, Vidallon ML, Mendoza DJ, Reyes CT. Health-promoting bioactivities of betalains from red dragon fruit (*Hylocereus polyrhizus* (Weber) Britton and Rose) peels as affected by carbohydrate encapsulation. J Sci Food Agric 2016;96:4679-89.
31. Martinez RM, Longhi-Balbinot DT, Zarpelon AC, Staurengo-Ferrari L, Baracat MM, Georgetti SR, *et al*. Anti-inflammatory activity of betalain-rich dye of *Beta vulgaris*: Effect on edema, leukocyte recruitment, superoxide anion and cytokine production. Arch Pharm Res 2015;38:494-504.