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## Research Article

# Characterization of Chemical Constituent and Evaluation of Antioxidant, Cytotoxicity, Potential of *Selenicereus hamatus* Crude Extract

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## Abstract

**Background and Objective:** The plant *Selenicereus hamatus* is a distinct species distantly related to the Grandiflora-complex, a species of Cactaceae and produces one of the largest flowers in the family of Cactaceae. The main aim of this study was to find out the antioxidant, cytotoxicity of the crude extract and to isolate, characterization of a chemical constituent of *Selenicereus hamatus*. **Materials and Methods:** The antioxidant of the methanol extract of *Selenicereus hamatus* was evaluated of its potential using DPPH and the cytotoxicity was evaluated using Brine shrimps (*Artemia salina*). Extraction, isolation and characterization of the isolated compound were done using methanol, CC, UV, TLC, GC-MS, NMR and FTIR. **Results:** The maximum potential of antioxidant was obtained from the isolated compound from the crude extract of *Selenicereus hamatus* of  $99 \mu\text{g mL}^{-1}$ , followed by the methanol crude extract of  $234 \mu\text{g mL}^{-1}$ . Cytotoxicity was obtained to mild in crude extract ( $58.71 \mu\text{g mL}^{-1}$ ) and high in the isolated compound of  $16.52 \mu\text{g mL}^{-1}$ . **Conclusion:** The study showed that the crude extract from *Selenicereus hamatus* has Neophytadiene and significant antioxidant and cytotoxicity activity which can be a potential agent to curtail the menace caused by pathogens and many more diseases like cancer. The pure compound was isolated for the first time in this plant extract as Neophytadiene.

**Key words:** Antioxidant, cytotoxicity, isolation, GC-MS, NMR, FTIR, extract, characterization, *Selenicereus hamatus*

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The family Cactaceae constitutes a well-defined lineage within the angiosperm order Caryophyllales Berchtold and J. Presl. The general understanding of evolutionary relationships in Cactaceae has improved in recent years as a result of molecular phylogenetic studies. However, parts of the Cactaceae phylogenetic tree remain to be resolved, in particular concerning the relationships of major clades and relationships at the species level<sup>1</sup>.

Many genera have been recognized as para or polyphyletic but limitations in taxon sampling, lack of statistical confidence of relevant nodes, or missing morphological analyses have so far prevented a consistent implementation of a phylogeny-based classification system. For the tribal and subtribal level, a revised classification for the whole family was proposed by Nyffeler and Egli<sup>2</sup>.

The biological classification *Selenicereus hamatus* belongs to the kingdom of Plantae and class of Magnoliopsida of the order of Caryophyllales. The plant belongs to the family of Cactaceae and the genus of Selenicereus thus the type *Selenicereus hamatus*. Cactaceae show complex patterns of convergent evolution in life forms, pollination syndromes and other traits and the obvious morphological characters and their states associated with these traits have frequently been used for diagnosing genera but they are often homoplastic

and genera based on those characters are therefore often shown as not monophyletic<sup>3</sup>.

*Selenicereus hamatus* is a cactus a member of the succulent plant family, they are often used as ornamental plants, but many are cultivated as crop plants, many of the cactus plants are native to North America, South America and West Indies. Many of the cactus plants grow as wild plants in arid and semi-arid regions in Africa and in other parts of the world, it forms an important part of the people's dietary equipment. The young leaves are used as vegetables, salads and the immature fruits for making gherkins<sup>4</sup>.

Cactus is a drought-tolerant due to its carbon dioxide fixation capacity (CO<sub>2</sub>). A cactus plant is normally situated to dry areas where it can be used as an alternative for food and fodder, as well as a live fence to protect agriculture fields<sup>5</sup>. It is also a high water use efficiency which is attributed to Crassulaceae Acid Metabolism (CAM) which is normally present in all cactus plant<sup>6</sup>. Cactus is commonly known as prickly pears having about 130 genera and 1,500 species of Cactaceae. Cactus exists in a wide range of shapes and sizes<sup>7</sup> of which *Selenicereus hamatus* happened to one.

*Selenicereus hamatus* is a distinct species distantly related to the Grandiflora-complex, a species of Cactaceae and produces one of the largest flowers (Fig. 1) in the family. It is a cultivated ornamental vine. It was reported to be a plant native of Mexico<sup>9,10</sup>.



Fig. 1: Leaves and flower of *Selenicereus hamatus*

The research objective aimed to extract, isolate and characterize the chemical constituent, as well as to evaluate the antioxidant, cytotoxicity, potential of *Selenicereus hamatus* crude extract

## MATERIALS AND METHODS

**Study area:** The study was carried out in Universiti Malaysia Sarawak from June 2016 to Jan 2020. The study lasted for six month.

**Chemical and equipment:** SIGMA the analytical solvent was used. Equipment from Shimadzu model QP 2010 GCMS, FTIR and JEOL NMR, DELTA version 5.0.4 software by JEOL. Jasco ultraviolet spectrophotometer model V-630.

**Plant material:** The aerial parts of *S. hamatus* was collected from Kota-Samarahan, Desa Ilmu, Lorong 10 Sarawak Malaysia in October 2019 to March 2020. The study lasted for six month.

**Methanol extract and fractions:** The air-dried sample of *S. hamatus* was grounded into powder, 500 g was extracted with methanol 96% (3 × 1 L) at room temperature in seven days and the residue was re-extracted for another seven days to obtain a total extract from the sample for easy isolation of good amount of the chemical constituents. The solvent was evaporated in a rotary evaporator and the obtained crude extract was kept in a vial for cytotoxicity, isolation and antioxidant studies<sup>12</sup>.

**Evaluation of antioxidant activity using DPPH (2, 2-diphenyl-1-picryl-hydrazyl):** The free radical scavenging assay of compound 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) was used to evaluate the antioxidant properties of the *S. hamatus* crude extract. The measurement was based on the method described by Umaru *et al.*<sup>13</sup>. The sample was prepared by diluting 6 mg of crude extract into 6 mL of methanol, producing a concentration of 1000 µg mL<sup>-1</sup>. The stock solution was sonicated to ensure the homogeneity of the sample. Three other concentrations were prepared at 10, 50 and 100 µg mL<sup>-1</sup>, diluted from the 1000 µg mL<sup>-1</sup> stock solution. Sample of 5000 µg mL<sup>-1</sup> was prepared separately by diluting 25 mg of crude extract into 5 mL of methanol.

Approximately 3 mL of 0.1 mM solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was each added into five series of prepared concentrations (10, 50, 100, 1000 and 5000 µg mL<sup>-1</sup>) of sample solutions (1 mL). The analysis was done in triplicate. The solution was mixed vigorously and left

to stand at room temperature for 30 min in the dark after which its absorbance was measured spectrophotometrically at 517 nm using Jasco ultraviolet spectrophotometer model V-630. Methanol was used as blank (only methanol) and negative control (1 mL methanol mixed with 3 mL DPPH), while ascorbic acid (vitamin C) as the standard. The concentration of the sample required to inhibit 50% of the DPPH free radical was calculated as IC<sub>50</sub> and the value was determined using the Log dose inhibition curve which performed by using PRISM version 3.02 software, based on the calculated values of the DPPH scavenging activity (%) of the sample<sup>16</sup>. DPPH scavenging activity (%) was calculated with formula:

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A<sub>0</sub> was the absorbance of the control, while A<sub>1</sub> was the absorbance in the presence of the sample<sup>14</sup>.

## Evaluation of *S. hamatus* Shrimp (*Artemia salina*) lethality test:

Evaluation of *S. hamatus* toxicity test against brine shrimp (*Artemia salina*) developed by McLaughlin, as reported by other studies<sup>15,16</sup>, was used in this study. Leached brine shrimp eggs were hatched in seawater and incubated for 48 hrs at 25°C. Exactly 3 mg of sample was dissolved in 3 mL methanol and the mixture was sonicated to ensure homogeneity of the extract. Four different volumes of 500, 250, 50 and 5 µL each from the stock solution was transferred into NUNC multidisc in triplicate. The solvent was allowed to evaporate under a running fume hood overnight and followed by the addition of 0.2 mL DMSO and 4.8 mL seawater to give a final concentration of 100, 50, 10 and 1 µg mL<sup>-1</sup>, respectively. Ten brine shrimp nauplii were transferred into each concentration in NUNC multi-disc and were observed every 6 hr for 24 hrs. The number of dead nauplii was calculated. Thymol was used as the positive control, whereas 0.2 mL DMSO and 4.8 mL seawater was used as the negative control. The data was analyzed to determine the concentration of the samples that kill 50% of brine shrimp at 24 hr or known as LC<sub>50</sub>. The LC<sub>50</sub> was calculated and determined by performing probit analysis in IBM SPSS Statistic<sup>16</sup> software of version 21.

**Isolation and purification:** Secondary metabolites in the crude extracts of *Selenicereus hamatus* were isolated and purified by using chromatographic methods namely column chromatography (CC), with thin layer chromatography (TLC) plate as a medium for visual identification.

**Thin Layer Chromatography (TLC):** Analytical thin-layer chromatography (TLC) was carried out by using an aluminum plate of 20×20 cm coated with silica gel 60 F254 (Merck 1.05554.0001). The TLC plate was cut into the smaller standardized size of 6.66 cm high and travel distance for the solvent and metabolites was set to 5 cm. The sample was spotted onto the TLC plate with a fine glass capillary tube and developed in a chromatographic developing chamber saturated by the vapor of suitable solvent systems at room temperature<sup>17,18</sup>. The plate was allowed to air-dry before observed under ultraviolet (UV) light. The separated components of the sample, which appeared as spots, were visualized under both short (256 nm) and long (360 nm) the wavelength of the UV light.

Reagent of vanillin dipping with 5% sulphuric acid in ethanol was used to detect the presence of certain natural products that did not appear in the earlier visualization under UV light. Vanillin dipping reagent was prepared by dissolving 6 g vanillin in 95 mL of 96% ethanol and 1.5 mL of concentrated sulphuric acid. The analysis was performed by spraying the vanillin dipping reagent on the TLC plate and allowed to air-dry. The TLC plate was then heated by using a hot plate at 150 °C to identify the presence of another natural product component, appear as colored spots. The  $R_f$  value of each component was determined<sup>18,19</sup>.

**Column Chromatography (CC):** Prior to the isolation process, the crude extract was examined on TLC, plate (Silica Gel 60 F254 Merck) to find the best solvent systems for column chromatography (Section 3.3.2.1). The solvent used was hexane, hexane-dichloromethane, dichloromethane, dichloromethane, chloroform, chloroform, chloroform-ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol. The basic principle of column chromatography is to separate a mixture of metabolites based on their molecular weight and polarity. A glass column of size 40/34 (large) was used for chromatography and the sorbent used was silica gel 60 (Merck 70-230 Mesh @ 0.0630.200 mm). Silica gel slurry was prepared by dissolving silica gel (150 g) with a suitable solvent, usually hexane. The column was prepared by pouring a slurry mixture of silica gel and solvent, into a glass column and allow it to settle down<sup>20</sup>. The packed column was left overnight before 4-10 g of sample was introduced onto the top of the packed column via a wet-packing method.

The column was eluted with suitable solvent systems with increasing polarity<sup>21</sup>. The column's valve was then opened and about 10 to 30 mL the fraction of the solvent coming out from the column was collected in test tubes<sup>19,22-23</sup>. The procedure was repeated by using different solvent systems, based on

increasing polarity. Samples from the column fractions were examined by using TLC plates in fewer suitable solvent systems to obtain the retention factor ( $R_f$ ) of any components that appeared as spots. Fractions with similar  $R_f$  values were combined<sup>22</sup>. Fractions which contain more than one components were further isolated and purified by using a smaller glass column of sizes 24/29 (medium) or 14/23 (small) with suitable solvent systems. The fraction with a single component (one spot) that appeared in the TLC plate was treated as a possible pure secondary metabolite. The combined fractions which contain the same single component were then allowed to air-dried or evaporated to dryness to obtain a pure secondary metabolite.

**Chemical structure elucidation:** Identification of the isolated secondary metabolite was made by various spectroscopy methods namely Gas Chromatography-Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR) and Fourier Transform Infra-Red spectrometry (FTIR) as described by Fasihuddin *et al.*<sup>21</sup>. The elucidation of chemical structures for the extracted secondary metabolite was made based on the data obtained from various spectroscopy methods and also the comparison with published information if available.

**Gas Chromatography-Mass Spectrometry (GC-MS):** Fractions from the column chromatography (CC) of one single spot on TLC were subjected for further analyses in GC-MS to obtain a single peak and a molecular per charge ( $m/z$ ) ratio. The GC-MS used was equipped with a BPX-5 column of 30 m length. With 0.25  $\mu\text{m}$  of film thickness and an internal diameter of 0.25 mm. the carrier gas used is helium at a constant rate flow of 1 mL per minute. One microliter of the pure sample was introduced in the GC-MS to obtain the required result; split mode of the ratio of 25:1 was used with an injection temperature set at 260 °C. The temperature of the oven used was programmed at 60 °C (isothermal for 5 min) with an increase of 10 °C per minute to 280 °C and ending with 10 min isothermal at 280 °C. At 70 eV, mass spectra were taken; a scan-interval of 0.5 sec and fragments from 45 to 450 Da<sup>19,23</sup>.

**Nuclear Magnetic Resonance (NMR):** Nuclear Magnetic Resonance (NMR) spectrometry was performed by using JEOL JNM-ECA 500 Spectrometer, based on the method as described by Umaru *et al.*<sup>23</sup>. The <sup>1</sup>H and <sup>13</sup>C spectra were measured at 500 and 125 MHz, respectively. The sample was dissolved in 0.8 mL chloroform D1 (CDCl<sub>3</sub>) and placed into NMR tube to make sample depth around 3.5 to 4 cm and ready to be analyzed by NMR spectrometer. Chemical shifts were reported as  $\delta$  units (ppm) and coupling constants (J) in

Hz. Integration of the  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  data was performed by using DELTA version 5.0.4 software by JEOL. Identification of the type of each  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  detected was based on the guide of the possible proposed structure given by NIST library.

**Fourier Transform Infra-Red Spectrometry (FTIR):** The chemical bonds (functional groups) of the compounds were detected by using Fourier Transform Infra-Red spectrometry (FTIR) (Thermo Scientific, Nicolet iS10 SMART iTR). The semi-solid, crystalline and powdered samples were introduced directly into FTIR. Scan range employed was from 400 to 4000  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$ , based on the method described by Umaru *et al.*<sup>23</sup>. The characteristic of the chemical bond was read by spectrum produced through transmittance of the wavelength of the light. The chemical bond in a molecule was detected by interpreting the infra-red transmittance spectrum.

## RESULTS AND DISCUSSION

The free radical scavenging potential of *S. hamacus* was calculated as  $\text{IC}_{50}$  and the value was determined using Log dose inhibition curve which performed by using PRISM version 3.02 software, based on the calculated values of the DPPH scavenging activity (%) of the sample. Table 1 demonstrated the antioxidant potential of the crude extract from *S. hamacus*. Thus exposure to the proton radical scavengers of the crude extract of *S. hamacus* will significantly decrease its characteristic absorption at 510 nm as reported by other study<sup>24,25</sup>, which gives the antioxidant properties of the sample as shown in Table 1. The result indicates the antioxidant properties of the plant *S. hamacus* which was determined from the  $\text{IC}_{50}$  value based on the percentage of DPPH scavenging activity as shown in Table 1. The  $\text{IC}_{50}$  value of methanol crude extract and the isolated compound were 234 and 99, respectively. This shows that the crude extract and the chemical constituent of the plant *S. hamacus* indicated a good antioxidant property when compared to the standard of  $\text{IC}_{50}$  (2.73  $\mu\text{g mL}^{-1}$ ).

The cytotoxicity of the crude extract and the isolated compound as shown in Table 2 and 3 at four different concentrations of crude extract and that of Neophytadiene showed strong cytotoxicity against brine shrimp with an  $\text{LC}_{50}$  value of 58.71 for the crude extract. At higher concentration of 100  $\mu\text{g mL}^{-1}$ , the methanol crude extract caused a number of  $8 \pm 0.56 \mu\text{g mL}^{-1}$  death of the brine shrimp as shown in Table 2, or an average of 85% whereas at a lower concentration of 10  $\mu\text{g mL}^{-1}$  the death rate was

$2 \pm 1.16 \mu\text{g mL}^{-1}$  or an average of 21% when compared to the positive standard of 1.16  $\mu\text{g mL}^{-1}$ . The cytotoxicity of the isolated compound was observed to be 16.52  $\mu\text{g mL}^{-1}$ . At higher concentration, the total number of death was observed to be  $10 \pm 0.00$  or 100% and a lower concentration of 10  $\mu\text{g mL}^{-1}$  of  $10 \pm 0.56 \mu\text{g mL}^{-1}$ . The result obtained when compared with the report of Moshi<sup>26</sup> which state that if the test sample showed  $\text{LC}_{50}$  between 30 to 100  $\mu\text{g mL}^{-1}$ , is considered to be mildly toxic, whereas when the  $\text{LC}_{50}$  is more than 100  $\mu\text{g mL}^{-1}$  is considered as being practically low or non-toxic. With this guideline, the result of this study showed that the crude extract (58.71  $\mu\text{g mL}^{-1}$ ) is considered mildly toxic. While Neophytadiene was considered to be toxic (16.52  $\mu\text{g mL}^{-1}$ )

**Purification and structural elucidation:** The isolated compound was obtained from the crude extract. Approximately 20 g of the crude extract was used for column chromatography, packed with silica gel using the dry-packed method. The column was eluted several times with solvent system hexane: ethyl acetate (7:3). The collected fractions in the test tube were analyzed using thin-layer chromatography (TLC) and viewed under ultraviolet (Uv) light followed by staining with vanillin solution as a maker. Fractions that showed fewer spots with similar  $R_f$  values on the TLC were combined and subjected to further isolation and purification. The first combined fraction obtained from the isolation of methanol crude extract was in yellowish. After further analyses a single spot was observed under UV light and was subjected to GC-MS, the single spot produced one peak at a retention time of 15.89 min this shows that the sample obtained is a pure compound. Figure 2 shows the gas chromatography of the isolated compound.

The physical appearance of the isolated compound was a light yellow powder with a weight of 9.46 mg. The compound has a similarity to the suggested structure by NIST library with a mass spectrum as a comparison, having the same base peak at  $m/z$  278, with a molecular weight of 278, which corresponded to a molecular formula of  $\text{C}_{20}\text{H}_{38}$ .

The IR spectrum of the isolated compound suggests that the chemical structure contains  $\text{CH}$ ,  $\text{CH}_2$  and  $\text{CH}_3$ . A signal was observed at an absorption band of 2973  $\text{cm}^{-1}$  indicating the presence of the C-H bond functional group. Whereas an

Table 1: Free radical scavenging activity against DPPH radical of *S. hamacus* crude extract

Plant	Extract and isolated compound	$\text{IC}_{50}$ ( $\mu\text{g mL}^{-1}$ )
Aerial part (leaf)	Methanol crude extract	234
Pure compound	Neophytadiene	99
Standard	Ascorbic acid	2.73

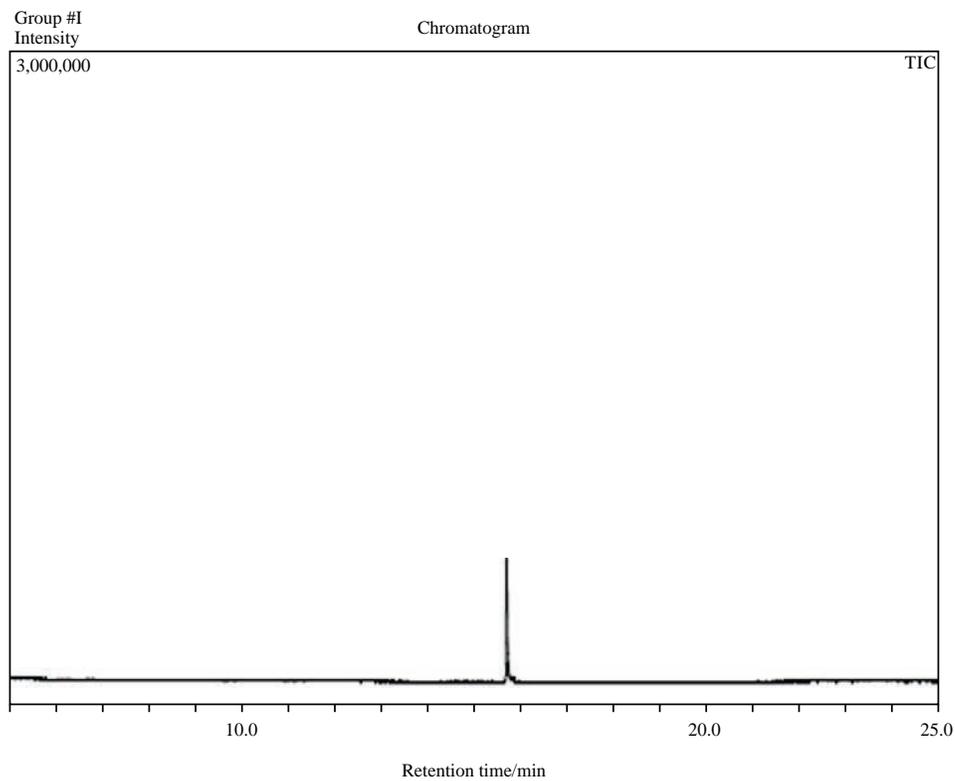


Fig. 2: Gas chromatography of the isolated compound

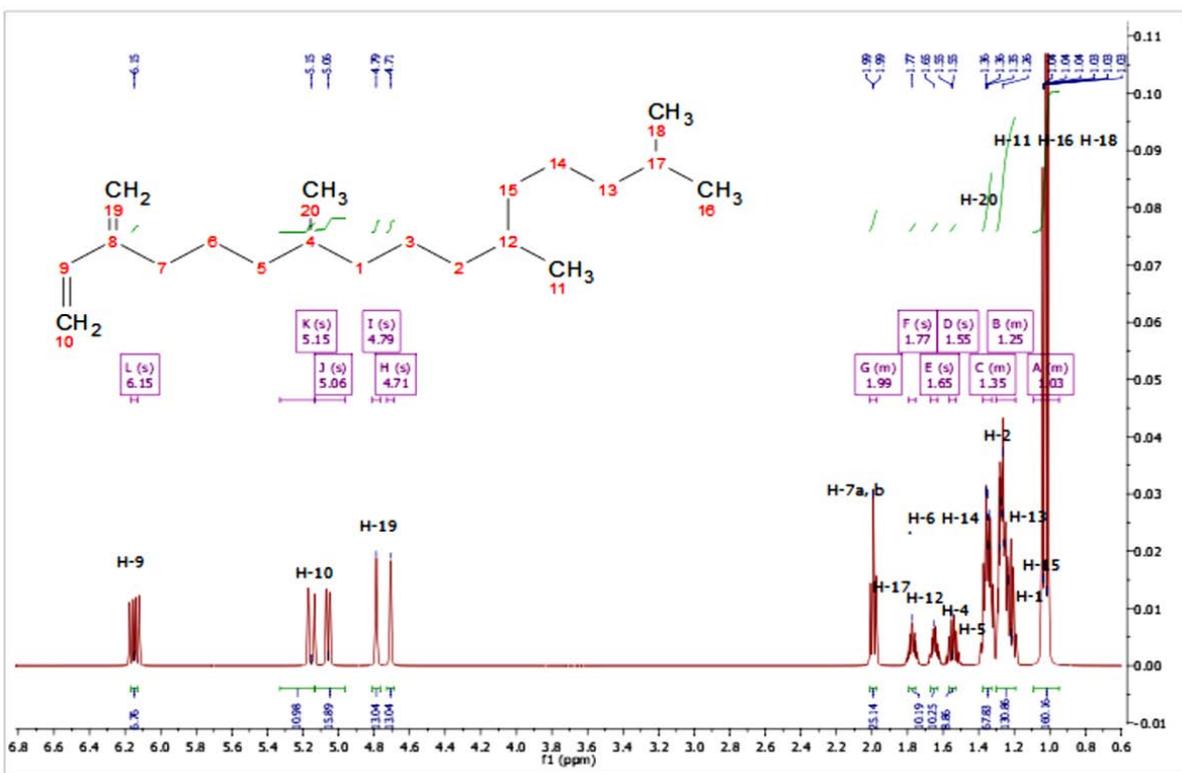


Fig. 3: <sup>1</sup>H-NMR spectrum of isolated compound from 0.6 to 6.8 ppm (500 MHz, CDCl<sub>3</sub>)

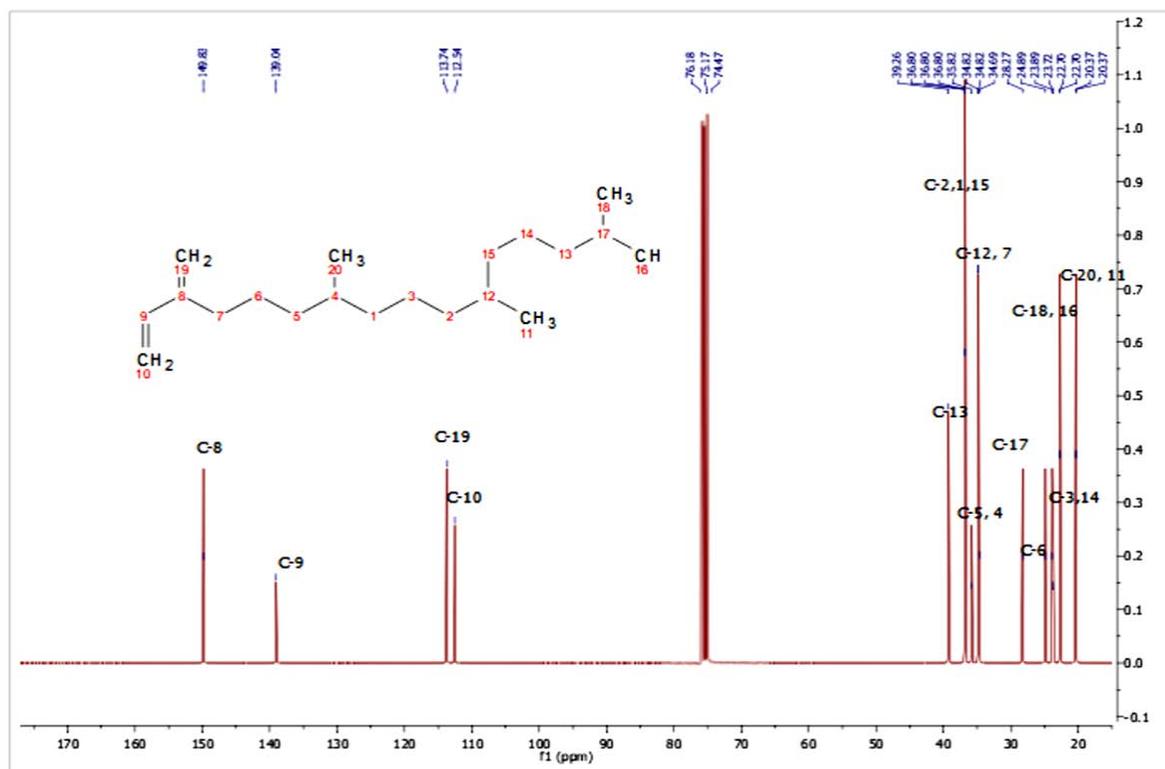


Fig. 4:  $^{13}\text{C}$ -NMR spectrum of the isolated compound from 20 to 170 (125 MHz,  $\text{CDCl}_3$ )

Table 2: Average death of *Artemia salina* at different concentrations of crude extract of *S. hamacus*

Crude extract	Average death of <i>Artemia salina</i> (Concentration ( $\mu\text{g mL}^{-1}$ ))				$\text{LC}_{50}$ ( $\mu\text{g mL}^{-1}$ )
	1	10	50	100	
Methanol	0	$2 \pm 1.16$	$4 \pm 1.24$	$8 \pm 0.56$	58.71
Negative control	0	0	0	0	-
Positive control (Thymol)	$5 \pm 0.56$	$7 \pm 0.57$	$10 \pm 0.00$	$10 \pm 0.00$	1.16

illustrated absorption was also observed at  $1517\text{ cm}^{-1}$  and  $1546$  of the IR spectrum indicating ethylene and  $1024\text{ cm}^{-1}$  indicating the presence of C-C bonds respectively.

Further identification of the isolated compound was performed by NMR analysis with integration and assignment of every proton NMR to the proposed chemical structure is based in the Table of  $^1\text{H}$ -NMR characteristics absorption and  $^1\text{H}$ -NMR peaks splitting pattern reported in Organic Chemistry by Janice as reported by Gerothanassis<sup>26</sup>. The result of proton NMR analysis is shown in Fig. 3 and 4. The  $^1\text{H}$ -NMR spectrum of the isolated compound shows a total of 24 proton resonance. A multiplet proton was observed at 1.28 (2H, m), 1.35 (2H, m), 1.36 (1H, m), 1.55 (2H, m), 1.99, 1.99 (2H, m), 1.36 (2H, m), 6.15 (1H, m), 5.15, 5.06 (2H, m), 1.03 (2H, m), 1.27 (2H, m), 1.36 (2H, m), 1.26 (2H, m), 1.03 (3H, m), 4.79, 4.71 (2H, m) and 1.04, 1.4, 1.04 (3H, m) and were assigned to H-2, H-3, H-4, H-5, H-6, H-7a,b, H-9, H-10a,b, H-11, H-13, H-14, H-15, H-18,

H-19a,b and H-20abc, respectively. A signal of singlet was observed at  $\delta$  1.65 (1H, s) and 1.99 (1H, s) as a C-H bond was assigned to H-12 and H-17. At the upfield region, two doublet proton chemical shift at  $\delta$  1.26 (2H, d) and  $\delta$  1.03 (3H, d) indicated the presence of five protons which are assigned to H-1 and H-16 indicating the presence of methylene in the structure of the isolated compound.

The assignment of every carbon of the isolated compound to the proposed chemical structure is also based on the table of  $^{13}\text{C}$ -NMR characteristics absorption reported in Organic Chemistry by Janice as reported by Gerothanassis<sup>26</sup>. The result showed 20 carbon resonance in the  $^{13}\text{C}$ -NMR spectrum of the isolated Compound as shown in Fig. 4 and Table 4. A peak signal was observed at the downfield of 149.83, 139.04, 112.54 and 113.74. They were assigned to C-8, C-9, C-10 and C-19. At the upfield region, 16 carbon signals were observed at chemical shift  $\delta$  36.80, 36.80, 23.72, 34.82,

Table 3: Average death of *Artemia salina* at different concentrations of Neophytadiene of *S. hamacus*

Isolated compound	Average death of <i>Artemia salina</i> (Concentration ( $\mu\text{g mL}^{-1}$ ))				$\text{LC}_{50}$ ( $\mu\text{g mL}^{-1}$ )
	1	10	50	100	
Neophytadiene	0	$2 \pm 1.00$	$10 \pm 0.56$	$10 \pm 0.00$	16.52
Negative control	0	0	0	0	-
Positive control (Thymol)	$5 \pm 0.56$	$7 \pm 0.57$	$10 \pm 0.00$	$10 \pm 0.00$	1.16

Table 4: Proton NMR signal of compound 1

$^1\text{H-NMR}$ assigned to Neophytadiene	$^1\text{H-NMR}$ chemical shift (ppm) of Neophytadiene	$^{13}\text{C-NMR}$ assigned to Neophytadiene	$^{13}\text{C-NMR}$ chemical shift (ppm) of Neophytadiene
H-1	1.26 (2H, d)	C-1	36.80
H-2	1.28 (2H, m)	C-2	36.80
H-3	1.35 (2H, m)	C-3	23.72
H-4	1.36 (1H, m)	C-4	34.82
H-5	1.55 (2H, m)	C-5	35.82
H-6	1.36 (2H, m)	C-6	24.89
H-7 <sup>ab</sup>	1.99, 1.99 (2H, m)	C-7	34.69
H-8	-	C-8	149.83
H-9	6.15 (1H, m)	C-9	139.04
H-10 <sup>ab</sup>	5.15, 5.06 (2H, m)	C-10	112.54
H-11	1.03 (2H, m)	C-11	20.37
H-12	1.65 (1H, s)	C-12	34.82
H-13	1.27 (2H, m)	C-13	39.26
H-14	1.36 (2H, m)	C-14	22.70
H-15	1.26 (2H, m)	C-15	36.80
H-16	1.03 (3H, d)	C-16	22.70
H-17	1.77 (1H, s)	C-17	28.27
H-18	1.03 (3H, m)	C-18	23.89
H-19 <sup>ab</sup>	4.79, 4.71 (2H, m)	C-19	113.74
H-20 <sup>abc</sup>	1.04, 1.04, 1.04 (3H, m)	C-20	20.37

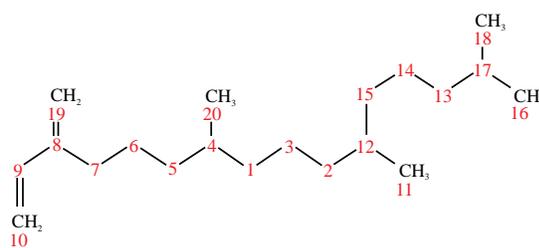


Fig. 5: Shows the structure of isolated compound Neophytadiene

35.82, 24.89, 34.69, 20.37, 34.82, 39.26, 23.70, 36.80, 22.70, 28.27, 23.89 and 20.37. They were all assigned to C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-11, C-12, C-13, C-4, C-15, C-16, C-17, C-18 and C-20, respectively.

The chemical shift of every proton and carbon NMR for the isolated Compound is showed in Table 4. Furthermore, based on spectroscopic data of the isolated Compound which include similarity of the mass spectrum with the suggested structure by NIST library, it can be concluded that the isolated Compound can be identified as Neophytadiene with a chemical formula  $\text{C}_{20}\text{H}_{38}$  as shown in Fig. 5.

Neophytadiene was reported to have a Carminative, Gastrin inhibitor, Antiulcerative and Histamine release

inhibitor, it as well as the potential of Antiprotozoal (Leishmania) and Antiparasitic<sup>27</sup>. Neophytadiene was reported to be antipyretic, analgesic and anti-inflammatory, antimicrobial, antioxidant<sup>28</sup>. The compound Neophytadiene was reported to have anti putrefying<sup>29</sup> and antimicrobial effects<sup>30</sup>.

## CONCLUSION

In conclusion, the study that the crude extract and the isolated pure compound from *Selenicereus hamatus* show antioxidant potential and thus indicated a significant protective effect against radical scavengers. Further, the toxicity effect of the isolated compound indicated that the Neophytadiene could be a very good agent to halt the effect of cancer suggesting its pharmaceutical potential for various ailments and diseases.

## SIGNIFICANCE STATEMENT

There is important information on the potential of this crude extract, the study discovers that the crude extract of *Selenicereus hamatus* and the chemical constituent isolated has a potential of good antioxidant and cytotoxicity

responsible for preventing and inhibiting the deleterious consequences of oxidative stress which has been reported to be among the major causative factors in the induction of many chronic degenerative disorders. Thus this study will help the researchers to uncover the critical areas of isolated chemical constituents that many researchers were not able to explore. This is a new theory on *Selenicereus hamatus* plant that may be explored.

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