IJPSR (2021), Volume 12, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 May 2020; received in revised form, 04 September 2020; accepted, 15 September 2020; published 01 May 2021

# PHYTOCHEMICAL PROFILING AND ANTICANCER ACTIVITY OF DRAGON FRUIT *HYLOCEREUS UNDATUS* EXTRACTS AGAINST HUMAN HEPATOCELLULAR CARCINOMA CANCER (HEPG-2) CELLS

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**Keywords:**

Dragon fruit, Phytochemicals, HepG-2, MTT, DAPI, GC-MS

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**ABSTRACT:** To investigate the phytoconstituents and anticancer potential followed by apoptotic studies of *H. undatus* fruit extracts on HepG-2 cells. Qualitative phytochemical profiling and anti-proliferation activity of Aqueous, Chloroform, Ethyl acetate, Hexane, and Methanol extracts of dragon fruit pulp was carried out. Based on the results of anti-proliferation studies, methanol extract was taken further for apoptotic study by treating HepG-2 cells for nuclear staining using DAPI and GC-MS analysis to elucidate the presence of active compounds. Qualitative phytochemical analysis revealed the presence of carbohydrates, tannins, saponins, anthocyanin, quinones, cardiac glycosides, terpenoids, triterpenoids, phenols, acids, and steroids in aqueous fruit extract. Similarly, chloroform fruit extract showed the presence of carbohydrates, saponins, alkaloids, cardiac glycosides, triterpenoids, phenols, and coumarins. Likewise, Ethyl acetate fruit extract had carbohydrates, tannins, saponins, flavonoids, alkaloids, anthocyanin, cardiac glycosides, terpenoids, triterpenoids, acids, and steroids. In hexane fruit extract, carbohydrate, saponins, anthocyanin, quinones, phenols, and acids were present. But more phytocompouds such as carbohydrates, tannins, saponins, flavonoids, alkaloids, anthocyanin, cardiac glycosides, terpenoids, triterpenoids, phenols, acids, and steroids were found in methanol fruit extract. A dose-dependent anti-proliferative assay revealed that the $IC_{50}$ value was 112.43 µg/ml in aqueous extract, 99.13 µg/ml in chloroform extract, 102.68 µg/ml in ethyl acetate extract, 83.96 µg/ml in hexane extract, and 69.09 µg/ml in methanol extract at 24 h incubation. Methanol fruit extract of *H. undatus* showed intense fragments of a nucleus as signs of apoptosis by DAPI staining. GC-MS results revealed that 43 aromatic compounds are present in methanol extract, which may have polyphenolic compounds. This study *in-toto* pragmatically shows that methanol extracts of *H. undatus* have demonstrated promising anticancer properties against human liver cancer (HepG-2) cells.

**INTRODUCTION:** Cancer is a major public health problem, with significant associated death and disability. It is the second leading cause of death in developed countries [1, 2]. Cancer is considered a human tragedy, and the causality prevalence resulting from cancer is increasing. WHO has predicted that by 2020, that the number of cancer new cases will reach 15 million [3]. Cancers that arise in any other part of the body, such as lung, colon, or breast, and spread to the liver is called metastatic cancer rather than liver cancer [4].

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DOI: 10.13040/IJPSR.0975-8232.12(5).2770-78

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(5).2770-78

Liver cancer is the second most common cause of cancer death, accounting for more than 7,00,000 deaths every year. Hepatocellular carcinoma (HCC) is the major type of liver cancer (70%-80%), followed by intrahepatic cholangio-carcinoma [5-11]. The main risk factors for liver cancer are hepatitis B/hepatitis C virus infection, alcohol consumption,

aflatoxin $B_{1,}$ and metabolic disorders [12]. Herbal drugs include plants, herbal complexes and herbal products or plant or even a combination of plants which were used thousand years before inventing modern drugs [13]. Herbal treasure options or complementary alternative medicine (CAM) offers a host of new phytochemicals that could be helpful as a preventive and clinical in managing the liver associated imbalance involving HCC. Several food items, as well as herbs that we use in our every-day life, could be protective agents against liver cancer. Studies have shown that traditional medicines could delay tumour progression, increase survival, and improve the quality of life due to synergistically efficient chemotherapy / radiotherapy [14].

Dragon fruit is an important source of phytochemicals such as polyphenols, flavonoids, and vitamin C, which are related to its antioxidant activity [15, 16]. The red and white dragon fruits especially have recently drawn growing attention worldwide not only because of their economic values, but also for their health benefits [17]. Red dragon fruit consumption was reported to decrease total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) levels while increasing the high-density lipoprotein cholesterol (HDL-C) level in type 2 diabetic [18]. Though both red and white dragon fruits are reported to be a rich, natural and cost-effective source of bioactive nutrients, few studies focused on the beneficial effects of white dragon fruit on diabetes and NAFLD (Non-alchoholic Fatty Liver Disease) [19]. Therefore, the present investigation was aimed to explore the phytoconstituents present in various pulp extracts of dragon fruit, *H. undatus*, and its anti-proliferative effect against human hepatocellular carcinoma (HepG-2) cell.

## MATERIALS AND METHODS:

**Collection and Identification:** Dragon fruits, *Hylocereus undatus* were collected from Koyambedu fruit market, Chennai, Tamil Nadu, India. The fruit was identified and authenticated as per morphological characteristics by Prof. P. Jayaraman, Institute of Herbal Botany, Plant Anatomy Research Centre, West Tambaram, Chennai, Tamil Nadu, India (PARC/2017/3377).

**Cancer Cell Line and Chemicals:** Cancer cell line HepG-2 (Human Hepatocellular Carcinoma cell) was purchased from National Centre for Cell Science (NCCS) Pune, India. Dulbecco's Modified Eagle Medium (DMEM), Trypsin- EDTA, Fetal Bovine Serum (FBS), 3-(4,5- Dimethyl thiazol-2yl)-2,5-dimethyl tetrazolium bromide (MTT), Dimethyl Sulphoxide (DMSO), Sodium bicarbonate, Propidium Iodide, Acridine Orange, Ethidium Bromide and Antibiotic solution were purchased from Sigma, Lout., USA. Likewise, 96 well plates, 6 well plates, tissue culture flasks (25 $mm^2$ and 75 $mm^2$), 15 ml and 50 ml centrifuge tubes were purchased from Hi-Media, USA. Chemicals used in the present study were extra pure and highest analytical grade.

**Preparation of Fruit Extracts:**

**Aqueous Extraction:** The outer epicarp of the fruit was separated, and the endocarp was shade-dried. Exactly 20 g of dried endocarp was crushed to powder by maceration method using a kitchen blender. A suspension of 5% (w/v) was prepared in a flask by adding hot boiled distilled water to the fruit powder and kept in a shaker at 200 rpm for 4 h at 37ºC. Then, the suspension was brought to room temperature, filtered through four layers of No.1 Whatman filter paper, and finally passed through a 0.22 µm filter. The filtered aqueous extract was freeze-dried, and the powder was stored at -20 ºC until further use. For cell culture studies, 10 g of the freeze-dried powder was taken and dissolved in DMSO [10].

**Chloroform, Ethyl Acetate, Hexane and Methanol Extraction:** The dried endocarp powder (20 g) was taken, and a suspension of 5% (w/v) was prepared in a separate flask by soaking the fruit powder in chloroform, ethyl acetate, hexane, and methanol.

They were kept in 4 h on a shaker, filtered, and evaporated at room temperature in petri-dishes. The dried material was retrieved and stored in tubes at -20ºC until further experimental uses. The chloroform, ethyl acetate, hexane, and methanol extracts were dissolved in DMSO to prepare (10 mg/ml) stock solution for cell culture studies [10].

**Preliminary Phytochemical Screening:** All five fruit extracts were subjected to preliminary phytochemical screening for their phytoconstituents according to Kokate (1988) method.

The powdered extracts were dissolved in acetone and used for further phytochemical studies [20].

**Anti-Proliferative Activity (MTT Assay):** The anti-poliferative effect of all the five extracts of *H. undatus* against HepG-2 cancer cells was assessed by the MTT (3-(4, 5- dimethylthiazol-2yl)-2, 5-diphenyltetrazolium) method as described by Mosmann (1983) [21].

**Nuclear Staining (DAPI Staining Method):** Nuclear staining by using DAPI stain and viewing the apoptotic morphology of the HepG-2 cells under a fluorescence microscope, containing appropriate DAPI stain filters followed by the method of Jang *et al*. (2002) [22].

**GC-MS Analysis (Gas-Chromatography and Mass Spectroscopy):** Best anticancer activity was observed in methanol fruit extract when compared to other extracts of *H. undatus*, and hence the phytoconstituents of methanol fruit extract with its structure was assessed by GC-MS chromatogram.

The filtrate was analyzed for secondary metabolites by using GC MATE II GC-MS (Agilent). For this, 1.0 µl of the compound was injected through HP-5 capillary column, maintained at 220°C, and helium was used as carrier gas at a flow rate of 1.0 ml/min. After analysis, the compound was identified by matching with the structural library.

**Statistical Analysis:** The data of MTT assay with five replicates were subjected to statistical analysis, and the mean value along with its respective standard error was calculated. The per cent change between control and experimental value was calculated. The data were analyzed statistically using 'Two Way Analysis of Variance (ANOVA)'. The data together with tables and graphs/bar diagrams are presented in appropriate places in text [10].

## RESULTS:

**Yield of Extracts:** The powder of fruit pulp of *H. undatus* is highly soluble in distilled water, chloroform, ethyl acetate, hexane, and methanol. All the fruit extracts of *H. undatus* resembled a dark brown coloured paste.

**Preliminary Phytochemical Screening:** In our study, the analysis of phytoconstituents of *H. undatus* showed the presence of carbohydrate, tannins, saponins, anthocyanin, quinones, cardiac glycosides, terpenoids, triterpenoids, phenols, acids, and steroids in aqueous extract, carbo-hydrate, saponins, alkaloids, cardiac glycosides, triterpenoids, phenols and coumarins in chloroform extract, carbohydrate, tannins, saponins, flavonoids, alkaloids, anthocyanin, cardiac glycosides, terpenoids, triterpenoids, acids, steroids in ethyl acetate, carbohydrate, saponins, anthocyanin, quinones, phenols, and acids in hexane extract, carbohydrate, tannins, saponins, flavonoids, alkaloids, anthocyanin, cardiac glycol-sides, terpenoids, triterpenoids, phenols, acids and steroids in methanol extract **Table 1.**

**TABLE 1: PHYTOCHEMICAL SCREENING OF VARIOUS FRUIT EXTRACTS OF *H. UNDATUS***

| S. no. | Secondary Metabolites | Aqueous | Chloroform | Ethyl Acetate | Hexane | Methanol |
|---|---|---|---|---|---|---|
| 1 | Acids | + | - | ++ | + | ++ |
| 2 | Alkaloids | - | + | + | - | ++ |
| 3 | Anthocyanin | + | - | +++ | ++ | ++ |
| 4 | Carbohydrate | +++ | +++ | +++ | +++ | +++ |
| 5 | Cardiac glycosides | ++ | + | + | - | ++ |
| 6 | Coumarins | - | + | - | - | - |
| 7 | Flavonoids | - | - | + | - | ++ |
| 8 | Glycosides | - | - | -- | - | - |
| 9 | Phenols | + | + | - | + | ++ |
| 10 | Protein | - | - | - | - | - |
| 11 | Quinones | + | - | - | + | - |
| 12 | Saponins | + | ++ | +++ | + | +++ |
| 13 | Steroids | ++ | - | +++ | - | +++ |
| 14 | Tannins | + | - | +++ | - | ++ |
| 15 | Terpenoids | + | - | +++ | - | ++ |
| 16 | Triterpenoids | ++ | + | +++ | - | +++ |

+++ = Strongly present ++ = slightly present + = Present in trace amount - = absent.

**Anticancer Activity:** In our study, anti-proliferation of the cells was assessed by MTT assay for 24 h in all the fruit pulp extracts of *H. undatus*, and the data are presented in **Table 2** and **Fig. 1**.

The data revealed that anticabcer activity was seen in the HepG-2 cells when treated with different concentrations (25, 50, 75, and 100) of various fruit pulp extracts; the cell anti-proliferation being directly proportional to concentration. Per cent cell viability of HepG-2 cells was assessed for 24 h in all the fruit pulp extracts at varying concentrations. The control cells were 100% viable, and the viability decreased significantly with an increase in the concentration of the extracts. The per cent decrease in cell viability was indirectly proportional to the concentration of *H. undatus* pulp extracts.

Statistical treatment of the data by two-way ANOVA showed that all the values were significant at 5% level.

**TABLE 2: PER CENT CELL VIABILITY OF HepG-2 CELLS WHEN TREATED WITH VARIOUS FRUIT EXTRACTS OF *H. UNDATUS* AT 24 H EXPOSURE**

| Concentration | Aqueous | Chloroform | Ethyl Acetate | Hexane | Methanol |
|---|---|---|---|---|---|
| Control | 100 | 100 | 100 | 100 | 100 |
| 25 µg/ml | 93.07 ± 0.06* | 91.59 ± 1.19* | 89.02 ± 0.61* | 81.30 ± 1.34* | 79.46 ± 0.41* |
| | (-6.92) | (-8.41) | (-10.98) | (-18.70) | (-20.54) |
| 50 µg/ml | 82.07 ± 0.06* | 78.67 ± 0.72* | 77.90 ± 0.28* | 61.34 ± 0.04* | 62.72 ± 0.88* |
| | (-17.83) | (-21.33) | (-22.10) | (-38.66) | (-37.28) |
| 75 µg/ml | 71.02 ± 0.48* | 64.09 ± 0.79* | 62.33 ± 0.27* | 55.19 ± 1.29* | 46.06 ± 2.16* |
| | (-28.98) | (-35.91) | (-36.67) | (-44.81) | (-53.94) |
| 100 µg/ml | 56.98 ± 0.43* | 49.50 ± 0.95* | 51.29 ± 0.38* | 40.71 ± 0.61* | 24.89 ± 3.35* |
| | (-43.02) | (-50.51) | (-48.71) | (-59.29) | (-75.11) |

Values are mean ± SD. of five individual observations Values in parentheses are per cent change over control - Denotes per cent decrease over control * Denotes that values are significant at P<0.05.

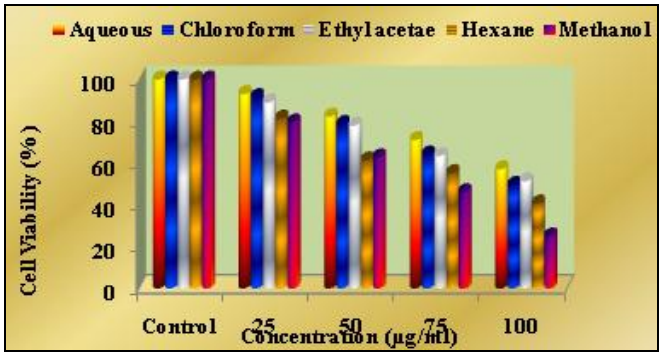


**FIG. 1: BAR DIAGRAM SHOWING DECREASE IN PERCENT VIABILITY OF HepG-2 CELLS, WHEN TREATED WITH VARIOUS FRUIT EXTRACTS OF H. UNDATUS AT 24 H EXPOSURE**

The inhibitory concentration ($IC_{50}$) value was 112.43 µg/ml in aqueous extract, 99.13 µg/ml in chloroform extract, 102.68 µg/ml in ethyl acetate extract, 83.96 µg/ml in hexane extract and 69.09 µg/ml in methanol extract at 24 h incubation **Table 3**.

At the end of 24 h, methanol extract showed higher activity when compared with the other four extracts, which obviously shows that methanol fruit extract has a profound effect in controlling HepG-2 cell proliferation even at low concentration.

**TABLE 3: $IC_{50}$ VALUES OF HepG-2 CELLS WHEN TREATED WITH VARIOUS FRUIT EXTRACTS OF H.UNDATUS AT 24 H EXPOSURE**

| S. no. | Sample | $IC_{50}$ Value (µg/ml) |
|---|---|---|
| 1 | Aqueous | 112.43 |
| 2 | Chloroform | 99.13 |
| 3 | Ethyl acetate | 102.68 |
| 4 | Hexane | 83.96 |
| 5 | Methanol | 69.09 |

**Cytomorphological Changes:** The morphological changes of HepG-2 cancer cells were photographed and presented in **Fig. 2**.

The control HepG-2 cancer cells showed irregular confluent aggregates with rounded and polygonal cell morphology.

On the other hand, treatment of the cells with *H. undatus* fruit pulp extracts at 24 h of incubation resulted in the shrinkage of polygonal cells, and it appeared spherical in shape.

The cell shrinkage increased progressively, and it was dose and time-dependent. The rate of shrinkage was high in methanol extract than that of other extracts.

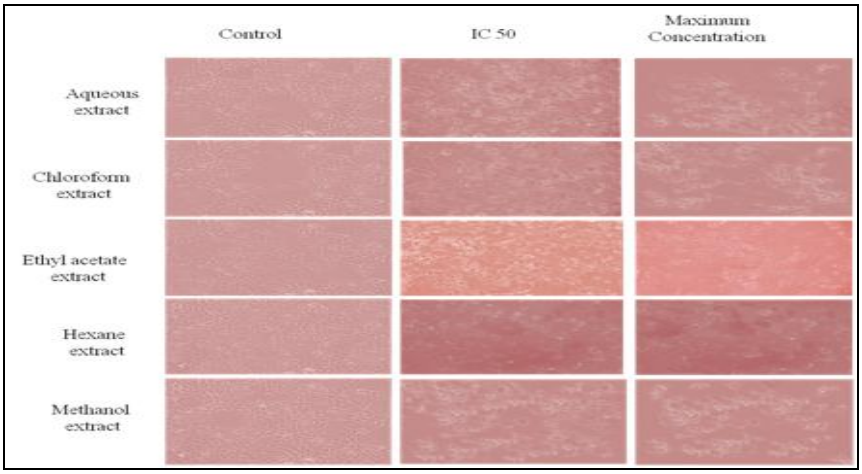


**FIG. 2: CELL MORPHOLOGY OF HepG-2 CELLS WHEN TREATED WITH $IC_{50}$ CONCENTRATIONS OF VARIOUS FRUIT EXTRACTS OF *H. UNDATUS* AT 24 H EXPOSURE**

**DAPI Staining:** To confirm whether the cytotoxic effect induced by methanol fruit extract of *H. undatus* involves apoptotic changes, the nuclear condensation was studied by the DAPI staining method. In the case of control cells, a very negligible number of positive cells were present. In case of cells treated with 69.09 µg/ml ($IC_{50}$ 24 h concentration) and 100 µg/ml (maximum concentration of methanol extract), a progressive increase in the number of the positive cell was observed **Fig. 3**. Methanol extract of *H. undatus* showed intense fragments of the nucleus as signs of apoptosis by DAPI staining.

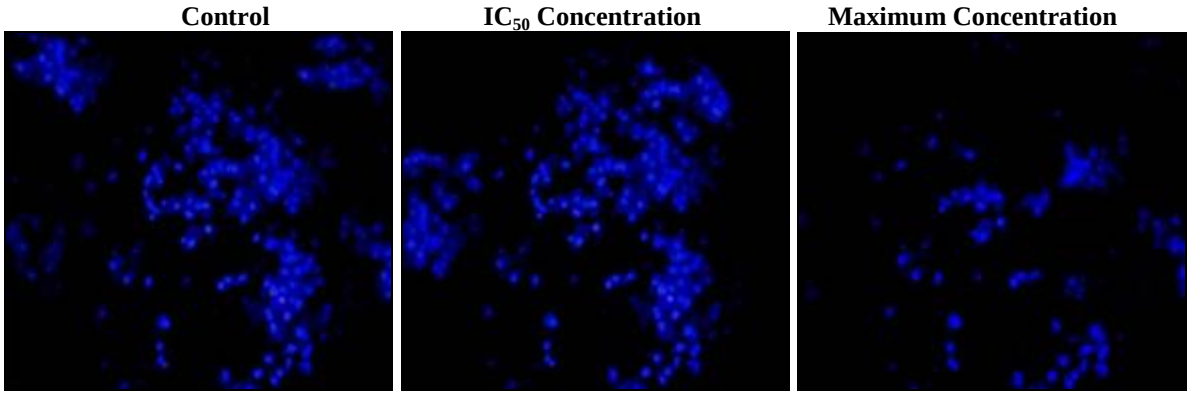


**FIG. 3: FLUORESCENCE MICROSCOPIC IMAGES OF HepG-2 CELLS, WHEN TREATED WITH 24 H $IC_{50}$ CONCENTRATION AND MAXIMUM CONCENTRATION OF METHANOL EXTRACT OF *H. UNDATUS***

**GC-MS Spectral Analysis:** The results pertaining to GC-MS analysis led to the identification of a number of compounds from the GC fractions of methanol extract of *H. undatus*. The presence of functional groups as detected by our GC-MS study of *H. undatus* methanol fruit extract revealed that it contains 43 active compounds with different retention time and mass peak such as n-Hexa-decanoic acid, sEthyl 13-methyl- tetra-deconaa, 3, 7, 11, 15 - Tetramethyl - 2 - hexa, Z-10-Methyl-11-tetradecen-1, n-Propyl 11-octa-decenate, Oleic acid, n-propyl 9,12-octadecadienoa, Ethyl 9,12,15-octadecatrieno, Methyl 19-methyl-eicosanate, Stigmasterol, Tricosanoic acid, methylest, Vitamin E, Pebtacosanoic acid, methyl, 17-Pentatriacotene, Squalene, N-(2-(Hydrohy (oxide) amino)-4, 4,7-Benzofurandione, -3-acetyl, Octacosyl triflora-oroacetate, Pregn-4-ene-3,11,20-trione, 4H, 8H - Benzo [1, 2 - b: 3, 4 - b] dip, Anthraerzo-statetraenol, 1,11-Dicartoethocy-1-beta, Silane, dimethyl (dimethyloct), 9,19-cydo-chloestene-, Ɔ-dio, Gamne-tocophenol, 4, 7-Benzo-flurandione, 3,H-cyloprop (1,2)-5. Alpha, Rhodoxanthin, Miibe-mycin b, 1-chloro-5-de, 2, 4, 6-Decatrienoic acid 'la', Hceadecananids. N, N-dis £ [2, Mitheamycin B, 5-demethoxy-5, d1-alpha-Tocophenol, 17-(1,5-dimethyl)-10,13, 1,1′-Dicarbo-ethyoxy-1. Beta, 4, 7-Benzofurandion, 1, 11-Benzofurandion, Campes-terol, 4, 25-Secoobscurinerran-4-one and 9, 19-Cycloeryost-24(28)-en-3 **Fig. 4.**

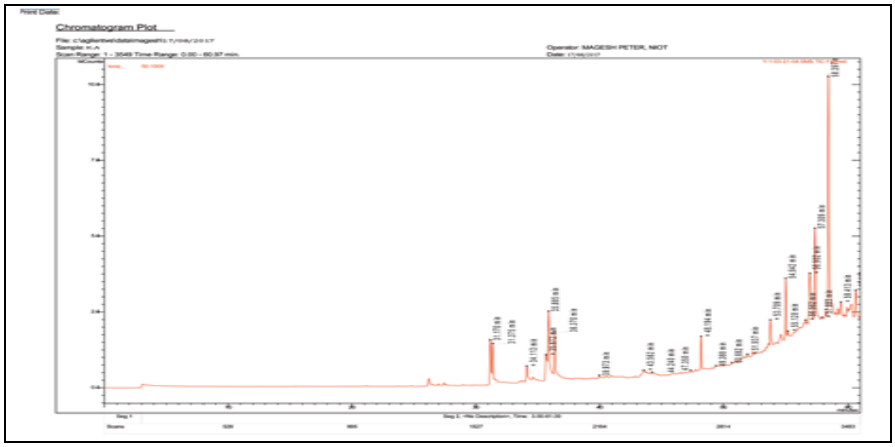

**FIG. 4: GC-MS CHROMATOGRAM OF *H. UNDATUS* METHANOL EXTRACT**

**DISCUSSION:** Fruits are an important source of natural phytocomponents with wide spectrum of applications. The 'greener' environmentally friendly processes in chemistry, and chemical technology is becoming increasingly popular and are much needed as a result of worldwide problems associated with environmental contamination. In Indian traditional medicine, *H. undatus* is used for diabetes treatment and prevention, to treat acne, to lower cholesterol levels, to improve cardiovascular health and to boost immunity. The use of this fruit is enormous and people of various countries are using this fruit for renal, bone disease and another ayurvedic purpose. The development of new phyto-compounds with a high impact on cancer therapy is a major challenge in medicine. Hence, the present investigation demonstrates that phytochemistry can conquer the limitation of conventional therapies used in practice.

In our study the analysis of phytoconstituents of *H. undatus* showed the presence of carbohydrate, tannins, saponins, anthocyanin, quinones, cardiac glycosides, terpenoids, triterpenoids, phenols, acids and steroids in aqueous extract, carbohydrate, saponins, alkaloids, cardiac glycosides, triter-penoids, phenols and coumarins in chloroform extract, carbohydrate, tannins, saponins, flavo-noids, alkaloids, anthocyanin, cardiac glycosides, terpenoids, triterpenoids, acids, steroids in ethyl acetate, carbohydrate, saponins, anthocyanin, quinones, phenols and acids in hexane extract, carbohydrate, tannins, saponins, flavonoids, alkaloids, anthocyanin, cardiac glycosides, terpenoids, triterpenoids, phenols, acids and steroids in methanol extract. Similar phytochemical analysis was done in *Emblica officinalis* [23], *Lycopersicon esculentum* [24], *Avicennia officinalis* and *Pleurotus pulmonarius* [25], *Momordica charantia* [26,] and *Garcinia mangostana* [7]. It has been reported that *H. undatus* consists of 150.46 ± 2.19 mg of polyphenolic component such as betalains, galic acid, and betacyanins per 100 g of dry weight [27]. In cacti, red-violet betacyanins and yellow betaxanthins are the most important fruit pigments, belonging to betalain pigment [28]. Betalain is a class of water-soluble pigments that provide color in a wide range of flowers and fruits [29]. Moreover, betacyanins that are attached to N-heterocyclic compounds are a class of compounds that can also be employed as antioxidants, with radical scavenging activities [30].

In our study, the $IC_{50}$ value was 112.43 µg/ml in aqueous extract, 99.13 µg/ml in chloroform extract, 102.68 µg/ml in ethyl acetate extract, 83.96 µg/ml in hexane extract, and 69.09 µg/ml in methanol extract at 24 h incubation. Similar results were also reported by several authors [7, 8, 10, 11, 31, 32]. Methanol extracts of the peels of dragon fruit showed anti-proliferative activity against AGS human gastric and MCF-7 breast cancer cells stronger than exhibited by the flesh extracts [33].

A positive correlation was found between peel and flesh content of polyphenols and flavonoids and their respective anti-proliferative activities. Negative correlations were found between the per cent cell viability of HeLa, AGS, and MCF-7 and the total polyphenol content [33]. Chiku and "dragon fruit" extracts exhibited remarkable inhibition of cell proliferation. The results were attributed to the scavenging of the cell proliferation-inducing nitric oxide by phytochemicals included in the fruit

extract, resulting in the inhibition of MCF-7 cell proliferation [34]. The anticancer properties of *Hylocereus* species were recently studied by several authors [33-35]. Several evidences showed that polyphenols, flavonoids, and betanins that present in the *Hylocereus* species are responsible for the anticancer effects [33-35]. It has been observed that *H. undatus* peel extracted by ethanol-water (50:50,v/v) solvent system showed anti-proliferative activity towards human hepatocellular carcinoma cell line (HepG-2) in a dose-dependent manner, and it recorded an $IC_{50}$ at 21.81 ± 0.01 mg/ml after 48 h of incubation [35].

The anticancer activity of our study also corroborates with the results of the above authors, thus finding support from their work. Polyphenols were believed to be the main phytochemical compound for such anti-cancer effect, although the exact compound is yet to be identified. The polyphenols act through scavenging nitric oxide (NO) free radicals that promote tumor vascularization and metastasis. Compounds that inhibited NO might be considered as potential anticancer agents. On the other hand, the presence of C2-C3 double bond and three adjacent hydroxyl groups in the flavonoids was also suggested to be crucial for anticancer effects [36-39]. Betacyanins that have a similar molecular structure as flavonoids were proposed to have similar anti-cancer effects [33].

The nuclear changes caused by methanol fruit extract of *H. Undatus*, involving apoptotic changes and the nuclear condensation was studied by the DAPI staining method. A very negligible number of positive cells were present in control cells, while in methanol extract treated cells both at $IC_{50}$ 24 h concentration and maximum concentration, a progressive increase in the number of the positive cell was observed. Methanol extract of *H. undatus* showed intense fragments of the nucleus as signs of apoptosis by DAPI staining. Work on DAPI staining was conducted in *Curcuma longa* on leukemic cell lines [37], thus supporting the present work. The GC-MS spectra are a useful tool in identifying the presence of certain functional groups in a molecule, and to confirm the types of functional groups on the biosorbent before and after chemical modification. The presence of glycosides, terpenoids, triterpenoids, phenols, and acids in GC-MS suggests that *H. undatus* methanol fruit extract is pharmacologically active, supporting the claim by traditional healers. This result obtained is comparable to the reported phytochemical components, which indicate the presence of polysaccharides, flavonoids, phenol, betacyanins in *H. polyrhizus*, *H. megalanthus*, and *H. undatus* [40-42]. It has been stated that phenolic acids constitute about one-third of the dietary phenols and they are present in free and bound forms [43]. Bound-phenolic may link to various plant components through ester, ether or acetal bonds [44]. The high content of polyphenols gives fruits remarkable antioxidant activity and may help lessen the risk of cancer [45]. The presence of phenols, betalains, and other antioxidant agents in dragon fruit might also be the reason for exerting antioxidant properties leading to the anti-proliferation of HepG-2 cells treated with methanol extract of dragon fruit. *In-toto*, our study investigated the anti-cancer activity of *H. undatus* fruit extract as a whole. The preliminary screening studies on phytochemical constituents of *H. undatus* fruit extract confirmed that the phytoconstituents present in these extracts possess the best anticancer and apoptotic activity. The present study indicates the anti-cancer potential of *H. undatus* fruit pulp methanol extract, and further experiments await the characterization of the active principle responsible for anti-cancer property. This study may be the subject of experimental validation and clinical trials to establish these said analogous as more potent drugs to treat various cancers in the future.

**CONCLUSION:** In conclusion, the methanol fruit pulp extracts of *H. undatus* have demonstrated promising anticancer and anti-apoptotic properties against human liver cancer (HepG-2) cells by an *in-vitro* method. Increasing awareness, promotion, and utilization of this fruit for public benefits are highly encouraged, and the identification of active phytoconstituents in the fruit pulp will serve as a natural cytotoxic agent against various cancers.

**ACKNOWLEDGEMENT:** The authors thank Prof. P. Jayaraman, Institute of Herbal Botany, Plant Anatomy Research Centre, West Tambaram, Chennai, Tamil Nadu, India, for authentic identification of dragon fruit, V CLIN BIO LAB Pvt. Ltd, Sri Ramachandra Medical University,

Porur, Chennai for cell line studies and NIOT Chennai for GC-MS analysis.

**CONFLICTS OF INTEREST:** The authors declare that there is no conflict of interest in this manuscript.

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**How to cite this article:**
Padmavathy K, Sivakumari K, Karthika S, Rajesh S and Ashok K: Phytochemical profiling and anticancer activity of dragon fruit *Hylocereus undatus* extracts against human hepatocellular carcinoma cancer (hepg-2) cells. Int J Pharm Sci & Res 2021; 12(5): 2770-78. doi: 10.13040/IJPSR.0975-8232.12(5).2770-78.