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PHYTOCHEMICAL PROFILE AND THERAPEUTIC VALUES OF PULP AND PEEL EXTRACTS OF *Hylocereus undatus*

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ABSTRACT

The present study was aimed to examine the therapeutic values of pulp and peel extracts of *Hylocereus undatus* by comparing the chemical compounds identified by using GC-MS. Antioxidant activities were measured using DPPH$^{\bullet}$, ABTS$^{\bullet+}$, superoxide radical, FRAP and phosphomolybdenum reduction assay. Antidiabetic and anti-inflammatory activities were determined using α-amylase inhibition method and heat induced hemolytic technique. The results showed that both the extracts reduced the oxidative stress with pulp extract showing stronger antioxidant activity. The antidiabetic and anti-inflammatory activities of pulp extract were greater than peel extract with IC_{50} values of 222.97 and 16.56 µg mL^{-1}, respectively. GC-MS analysis showed the presence of phytosterols, steroids, triterpene, phenolic compounds and esters of fatty acids. The study shows the presence of bioactive compounds in *H. undatus* pulp and peel extracts with promising health benefits.

Keywords: Antidiabetic, anti-inflammatory, antioxidant, *Hylocereus undatus*, oxidative stress

INTRODUCTION

Oxidative stress refers to the imbalance between the production of free radicals and the ability of body's natural defense system to detoxify them (Pizzino *et al.*, 2017). Oxi-inflammation is characterized by the presence of increased oxidative stress and chronic inflammation. Oxi-inflammation alters a series of biochemical reactions that occur in different organ systems. Further, oxi-inflammation markedly contributes to the development of cardiovascular disorders, urological diseases, cancer and complications associated with type 2 diabetes mellitus (Vikram *et al*., 2014). Factors leading to the oxidative stress include pollution, smoking, exposure to harmful radiations and inadequate consumption of fruits and vegetables (Tan *et al.*, 2018).

Regular intake of fruits is essential for normal physiological functioning and for reducing the risk of developing non-communicable diseases. Essential micronutrients and non-nutritive

secondary metabolites present in fruits modulate immune system, reduce endothelial dysfunction, decrease chronic inflammation and regulate the action of endogenous antioxidant enzymes (Carter *et al*., 2010; Boeing *et al*., 2012; Folchetti *et al*., 2014). Dragon fruits are rich in β-cyanins, β-xanthins, flavonoids, dietary polyphenols, vitamins and minerals (Nurul and Asmah, 2014). There are 3 species of dragon fruit namely *Hylocereus polyrhizus*, *H. undatus* and *H. guatemalensis*. *H. undatus* (Haw.) Britton & Rose, commonly known as white dragon fruit or white pitaya, is cultivated in Bangladesh, Indonesia, Malaysia, Taiwan, Sri Lanka and Vietnam (Choo *et al*., 2011). The flesh of the fruit is white in colour and contains numerous minute black seeds. *H. undatus* lowers lipid peroxidation, prevents platelet aggregation, reduces blood sugar levels, maintains gut microflora and exhibits antioxidant, anti-proliferative and hepatoprotective activity (Choo *et al*., 2016; Dasaesamoh *et al*., 2016; Sudha *et al*., 2017).

Inedible products obtained during the manufacture of different processed fruit products include peel, rind, seed and pomace. These waste substrates contain nutrients, volatile components, organic acids and essential fatty acids that can be productively utilized in different ways. Generally, dragon fruit peels constitute 22% of the whole fruit and are usually discarded (Nurliyana *et al*., 2010). Limited information is available on the therapeutic aspects of pulp and peel extracts of *H. undatus*. Hence, the objective of present study was to compare the protective effects of pulp and peel extracts of *Hylocereus undatus* against oxidative stress and to identify the chemical compounds involved using GC-MS analysis.

MATERIALS AND METHODS

Preparation of extract

Fresh white dragon (*Hylocereus undatus*) fruits were purchased from Koyambedu market Chennai, Tamil Nadu (India). The fruits were washed using tap water and finally rinsed with distilled water. The peels were removed manually using a sharp knife and cut into small pieces. Fresh pulp and peel of *H. undatus* (100 g each) were immersed separately in 200 mL ethanol and left in an orbital shaker for 24 h. The extracts were filtered through Whatman filter paper No. 1. After filtration, the solvent was removed using rotary vacuum evaporator at 40ºC. The resulting extract was stored at 4ºC and used for further analysis.

Qualitative analysis of phytochemicals

The extracts (pulp and peel, taken separately) were screened for the presence of different phytochemicals using standard methods (Raaman, 2006).

Test for alkaloids: The extract (50 mg) was mixed with 1 mL dilute HCl and filtered. To this filtrate, Mayer's reagent was added drop by drop and observed for the presence of creamy white precipitate.

Test for glycosides: To 2 mL extract, 3 mL chloroform was added and shaken. Later, the chloroform layer was separated, 10% ammonia solution added and observed for pink colour development.

Test for saponins: The extract (50 mg) was mixed with 10 mL distilled water, shaken vigorously for 5 min and observed for the presence of foam formation.

Test for phenols: The extract (50 mg) was mixed with 5 mL distilled water and 0.5 mL 5% ferric chloride solution added. A dark green colour indicated the presence of phenols.

Test for flavonoids: To 5 mL extract, 2 mL sodium hydroxide solution was added, and observed for the presence of yellow colour. Later, concentrated sulphuric acid was added drop by drop till the disappearance of yellow colour.

Test for terpenoids: To 5 mL extract, 2 mL chloroform was added. Presence of reddish brown colour layer was observed on addition of concentrated sulphuric acid.

Test for steroids: To 2 mL extract, 1 mL chloroform was added. Later, 0.5 mL acetic anhydride and 1 mL concentrated sulphuric acid were added for the formation of blue green colour.

Test for tannins: To 2 mL extract, a few drops of 1% gelatin solution and 10% NaCl solutions were added. The precipitation of gelatin indicated the presence of tannins.

Estimation of total phenol content (TPC): Briefly, the extract (100 µL) was mixed with 1 mL Folin Ciocalteu reagent and 1 mL of 20% Na_2CO_3. The mixture was incubated at 25ºC for 30 min. The absorbance was measured at 760 nm using an UV visible spectrophotometer (Elico 104 - Uvsar). TPC was expressed as µg gallic acid equivalent (GAE) mg^{-1} (Singleton *et al.*, 1999).

Estimation of total flavonoid content (TFC): Briefly, the extract (500 µL) was mixed with 0.5 mL $NaNO_3$ (5%) along with 0.3 mL $AlCl_3$ (10%) and the mixture was incubated at 25ºC for 5 min. Later, 1 mL 1 M NaOH was added and the reaction mixture incubated for 15 min. The absorbance was measured at 510 nm using an UV visible spectrophotometer. TFC was expressed as µg quercetin equivalent (QE) mg^{-1} (Chang *et al.*, 2002).

Antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging activity: Briefly, 1 mL 0.1 mM $DPPH^{\bullet}$ solution was added to different concentrations of pulp and peel extracts (50-300 µg mL^{-1}) separately. The absorbance was measured at 517 nm after 30 min of reaction in dark at 25ºC using an UV visible spectrophotometer. The results were expressed as % inhibition of $DPPH^{\bullet}$ free radical (Blois, 1958).

2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$ radical) scavenging activity: $ABTS^{\bullet+}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture allowed to stand in dark at 25ºC for 12-16 h. Different concentrations (10-60 µg mL^{-1}) of pulp and peel extracts were separately mixed with 1 mL diluted $ABTS^{\bullet+}$ solution and incubated for 10 min. The absorbance was measured at 734 nm using an UV visible spectrophotometer and the results were expressed as % inhibition of $ABTS^{\bullet+}$ free radical (Delgado and Morales, 2005).

Superoxide (O_2^-) radical scavenging activity: Different concentrations of pulp (20-120 µg mL^{-1}) and peel extracts (50-300 µg mL^{-1}) were separately mixed with 50 mM phosphate buffer (pH 7.4), 200 µL 12 mM EDTA, 200 µL 1.5 mM riboflavin and 100 µL 50 mM NBT. The reaction mixture was left inside an UV transilluminator for 15 min. Then the absorbance was measured at 590 nm using an UV visible spectrophotometer. The results were expressed as % inhibition of superoxide (O_2^-) anion (Lokesh *et al*., 2009).

Ferric (Fe^{3+}) reducing power assay: The test tubes containing different concentrations of pulp extract (20-120 µg mL^{-1}) and peel extract (50-300 µg mL^{-1}) were separately mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL 1% $K_3[Fe(CN)_6]$. The test tubes were covered with aluminum foil sheets and incubated at 50ºC for 20 min. After cooling, 1 mL 10% TCA and 1 mL 0.1% freshly prepared $FeCl_3$ were added. The absorbance was measured at 700 nm using an UV visible spectrophotometer and the results were expressed as absorbance value measured at 700 nm (Yen and Chen, 1995).

Phosphomolybdenum reduction assay: Different concentrations of pulp extract (20-120 µg mL^{-1}) and peel extract (50-300 µg mL^{-1}) were separately mixed with 1 mL reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The test tubes were capped with aluminum foil sheets and incubated in a water bath at 95°C for 90 min. After cooling, the absorbance was measured at 695 nm using an UV visible spectrophotometer and the results

were expressed as the absorbance values measured at 695 nm. Ascorbic acid was used as standard for all antioxidant assays (Prieto *et al*., 1999).

Antidiabetic activity

Briefly, 500 µL 0.02 M sodium phosphate buffer was added to different concentrations (50-300 µg mL^{-1}) of pulp and peel extracts separately. Then 10 µL α-amylase and 500 µL soluble starch (1%, w/v) were added and incubated for 1 h. The reaction was stopped by adding 100 µL 1 N dilute HCl. Finally, 200 µL freshly prepared iodine solution was added and the absorbance read at 565 nm using an UV visible spectrophotometer. The results were expressed as % inhibition of α-amylase. The standard solution used was acarbose (Kotowaroo *et al*., 2006).

Anti-inflammatory activity

Briefly, 200 µL RBC (10% suspension) was added to different concentrations of (20-120 µg mL^{-1}) pulp and peel extracts separately. The volume was brought to 1 mL using saline solution. The reaction mixture was incubated at 56ºC for 30 min. After cooling, the samples were centrifuged at 2500 rpm for 5 min and the absorbance measured at 560 nm using an UV visible spectrophotometer. The results were expressed as % inhibition of hemolysis and the standard used was aspirin (James *et al*., 2009).

GC-MS analysis

The extracts (pulp and peel, taken separately) were injected into a HP-5 column (30 m x 0.25 mm with 0.25 µm film thickness) [Agilent Technologies 6890 N JEOL GC Mate II GC-MS model]. Helium was used as carrier gas at a constant flow rate of 1 mL min^{-1} and the injector was operated at 200°C. The column oven temperature was maintained between 50 and 250°C at 10°C min injection mode. The following MS conditions were used: ionization voltage of 70 eV, ion source temperature 250°C, interface temperature 250°C and mass range 50-600 mass units. NIST database was used for interpreting the different functional volatile compounds present in pulp and peel extracts of *H. undatus* (Begam *et al.*, 2020).

Statistical analysis

The data were expressed as mean ± standard deviation of triplicate readings. The IC_{50} value was calculated using linear regression analysis.

RESULTS AND DISCUSSION

Phytochemicals possess numerous biological properties which include antioxidant property, antimicrobial effect, stimulation of immune system, decreasing platelet aggregation, regulation of hormone metabolism, anti-cancer, antidiabetic, antihyperlipidemic, anti-inflammatory and anti-thrombotic activities (Nyamai *et al*., 2016). In this study, phytochemicals such as alkaloids, terpenoids, phenols, flavonoids, glycosides, steroids, saponins and tannins were found present in both the extracts. Total phenol content of pulp extract (515.38 ± 1.09 µg GAE mg^{-1}) was higher than peel extract (335.77 ± 1.63 µg GAE mg^{-1}). Total flavonoid content of pulp and peel extracts were 93.10 ± 2.44 and 86.21 ± 2.44 µg QE mg^{-1}, respectively.

Persistent hyperglycemia coupled with oxidative stress and inflammation lead to excess production of reactive oxygen species and alters the body's natural defense antioxidant enzymes (Elmarakby *et al*., 2012; Donath *et al*., 2013). Free radicals or reactive oxygen species (ROS) are by-products of oxygen metabolism. Some of the free radicals produced in body include superoxide, nitric oxide, hydroxyl, peroxyl and alkoxyl radicals. They attack the body's essential biomolecules and eventually cause cell damage along with homeostatic disruption (Lobo *et al*., 2010).

Table 1: Antioxidant activities of pulp extract of *Hylocereus undatus*

Free radical scavenging assays					
DPPH· radical		$ABTS^{\bullet+}$ radical		Superoxide (O_2^-) radical	
Concentration (µg mL^{-1})	Inhibition (%)	Concentration (µg mL^{-1})	Inhibition (%)	Concentration (µg mL^{-1})	Inhibition (%)
50	3.96 ± 0.23	10	60.46 ± 0.74	20	46.86 ± 0.82
100	26.27 ± 0.80	20	74.59 ± 0.88	40	47.53 ± 1.04
150	31.84 ± 0.78	30	83.02 ± 0.63	60	52.69 ± 1.47
200	78.44 ± 0.66	40	91.44 ± 0.43	80	57.18 ± 0.42
250	84.02 ± 0.67	50	95.24 ± 0.65	100	58.97 ± 0.69
300	89.72 ± 0.89	60	98.78 ± 0.40	120	60.54 ± 0.63
Reducing power assays					
FRAP assay			Phosphomolybdenum assay		
Concentration (µg mL^{-1})	Absorbance @ 700 nm		Concentration (µg mL^{-1})	Absorbance @ 695 nm	
20	0.59 ± 0.01		20	0.62 ± 0.02	
40	0.72 ± 0.02		40	0.77 ± 0.01	
60	0.80 ± 0.04		60	0.81 ± 0.02	
80	0.83 ± 0.01		80	0.86 ± 0.04	
100	0.87 ± 0.03		100	0.88 ± 0.03	
120	0.89 ± 0.02		120	0.89 ± 0.04	

During oxidative stress, the amount of ROS produced in body increases radically and causes significant damage (Guzik *et al*., 2003). The harmful effects of free radicals can effectively and actively be blocked by antioxidants. Antioxidants are a group of biological molecules that can neutralize ROS by accepting or donating electron(s) and convert them into less stable products (Kumar, 2014). The present study signifies the defensive role of pulp extract of *H. undatus* against oxidative stress through its notable antioxidant activities.

It is evident that pulp and peel extracts of *H. undatus* exhibited potent ability to scavenge the free radicals (Tables 1 and 2). IC_{50} value refers to the concentration required to scavenge 50% of free radicals. Lower the IC_{50} value, greater was the activity (Table 3). Based on the IC_{50} values, the ability of pulp extract to scavenge the free radicals can be ranked in the order of $ABTS^{\bullet+}$> superoxide (O_2^-) anions> $DPPH^{\bullet}$.

Table 2: Antioxidant activities of peel extract of *Hylocereus undatus*

Free radical scavenging assays					
DPPH· radical		$ABTS^{\bullet+}$ radical		Superoxide (O_2^-) radical	
Concentration (µg mL^{-1})	Inhibition (%)	Concentration (µg mL^{-1})	Inhibition (%)	Concentration (µg mL^{-1})	Inhibition (%)
50	24.51 ± 1.33	10	23.38 ± 0.62	50	24.44 ± 0.23
100	45.79 ± 0.96	20	28.05 ± 0.72	100	28.07 ± 0.16
150	57.57 ± 1.46	30	33.34 ± 0.08	150	35.85 ± 0.32
200	60.68 ± 0.97	40	44.75 ± 1.87	200	41.48 ± 0.43
250	74.11 ± 1.33	50	46.12 ± 1.65	250	46.72 ± 0.55
300	75.47 ± 0.86	60	65.76 ± 0.68	300	63.07 ± 0.28
Reducing power assays					
FRAP assay			Phosphomolybdenum assay		
Concentration (µg mL^{-1})	Absorbance @ 700 nm		Concentration (µg mL^{-1})	Absorbance @ 695 nm	
50	0.15 ± 0.01		50	0.37 ± 0.01	
100	0.34 ± 0.02		100	0.47 ± 0.03	
150	0.44 ± 0.05		150	0.76 ± 0.04	
200	0.55 ± 0.03		200	0.79 ± 0.02	
250	0.57 ± 0.03		250	0.82 ± 0.01	
300	0.61 ± 0.01		300	0.88 ± 0.04	

Table 3: IC_{50} values ((µg mL^{-1}) of free radical scavenging assays as affected by peel and pulp extract of *Hylocereus undatus*

Samples	DPPH· radical	$ABTS^{\bullet+}$ radical	Superoxide (O_2^-) radical
Pulp extract	127.49	8.26	56.10
Peel extract	130.32	45.62	237.84
Ascorbic acid	8.61	4.21	5.29

The potential of peel extract to inhibit the action of free radicals was in the order of $ABTS^{\bullet+}$> $DPPH^{\bullet}$> superoxide (O_2^-) anions. The reducing power of a component is based on the absorbance value. Greater the absorbance, greater is the reducing power. Both the extracts showed strong reducing power. On comparing the overall antioxidant power, pulp extract had greater antioxidant activity. For pulp extract, the IC_{50} values for $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and superoxide (O_2^-) assay were 127.49, 8.26 and 56.10 µg mL^{-1}, respectively. Using $DPPH^{\bullet}$ assay, Susanti *et al*. (2012) reported the IC_{50} value of methanolic pulp extract of *H. undatus* to be 193 µg mL^{-1}. Halimoon and Hasan (2010) in their comparative study on antioxidative activity of white dragon fruit and green kiwi fruit stated white dragon fruit to be rich in natural antioxidants. Fidrianny *et al.* (2014) evaluated the potential of *H. polyrhizus*, *H. undatus* and *H costaricensis* peels to inhibit the action of free radicals using different solvent extracts. The results showed that the radical scavenging potential of dragon fruit peels ranged from 46.51 to 53.87% for $DPPH^{\bullet}$ assay; while for $ABTS^{\bullet+}$assay the values ranged between 55.96 and 72.47% with *H. undatus* peel extract functioning as the excellent free radical scavenger. In the present study, the antioxidative effect of peel extract in terms of IC_{50} value for $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and superoxide (O_2^-) assay was 13.32, 45.62 and 237.84 µg mL^{-1}. Ayub *et al.* (2019) examined the antioxidant activity of foliage and peel extracts of *H. undatus* and stated the promising role of *H. undatus* peels as natural antioxidants. Nurliyana *et al.* (2010) evaluated the antioxidative activity of dried pulp and peel extracts of *H. undatus* and *H. polyrhizus* and ranked the capacity to scavenge ROS in the order of *H. polyrhizus* peels > *H. undatus* peels > *H. polyrhizus* pulp > *H. undatus* pulp.

The present study indicated that the pulp extract possessed greater antidiabetic and anti-inflammatory activities as compared to the peel extract (Table 4). For antidiabetic assay, the IC_{50} values of pulp and peel extracts were 222.97 and 281.42 µg mL^{-1}, respectively. For anti-inflammatory activity, the IC_{50} value of pulp and peel extracts were 16.56 and 54.84 µg mL^{-1}, respectively. Evidence from the animal studies underlines the role of dragon fruit in preventing type 2 diabetes mellitus. Dragon fruit regenerates beta cells of pancreas, reduces resistance caused by fibroblast growth factor 21, attenuates inflammation and protects the body from harmful effects caused by free radicals (Swarup *et al*., 2010; Song *et al*., 2016). Antioxidants, micronutrients and phytochemicals present in dragon fruit contribute to various functional bioactivities. The pulp extract of *H. undatus* inhibited the process of inflammation and blocked the action of α-amylase in a dose dependent manner. Further, GC-MS analysis of pulp extract showed the presence of phytosterols, phenolic compounds, triterpene and esters of fatty acids. The presence of these phyto-

Table 4: Antidiabetic and anti-inflammatory activities of pulp and peel extracts of *Hylocereus undatus*

Antidiabetic activity			Anti-inflammatory activity		
Concentration (µg mL^{-1})	Inhibition (%) of α-amylase		Concentration (µg mL^{-1})	Inhibition (%) of hemolysis	
	Pulp extract	Peel extract		Pulp extract	Peel extract
50	9.76±0.03	7.84±0.02	20	60.37 ± 0.37	41.67 ± 0.95
100	14.53±0.14	21.85±0.03	40	62.87 ± 0.55	44.63 ± 1.25
150	39.41±0.11	28.58±0.04	60	82.04 ± 0.83	54.70 ± 1.09
200	49.45±0.12	37.52±0.08	80	82.87 ± 0.56	66.15 ± 1.21
250	56.06±0.13	46.95±0.04	100	83.89 ± 0.70	72.93 ± 1.28
300	56.69±0.08	53.30±0.05	120	84.73 ± 0.71	79.70 ± 1.35

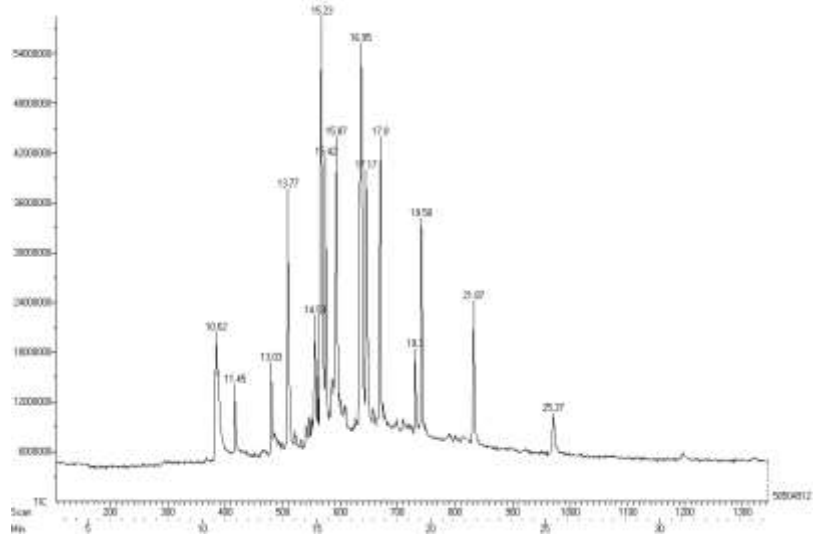

Fig. 1: GC-MS chromatogram of pulp extract of *Hylocereus undatus*

constituents contributes to the therapeutic values of dragon fruit. Clinical trials report the role of dragon fruit in treatment and prevention of type 2 diabetes mellitus. On the other hand, meta-analysis and systematic review by Poolsup *et al.* (2017) concluded that the results of clinical trials are inconsistent and do not significantly confirm the antidiabetic efficacy of dragon fruit. Therefore, more randomized clinical trials are required to prove the hypo-glycaemic effect of dragon fruit.

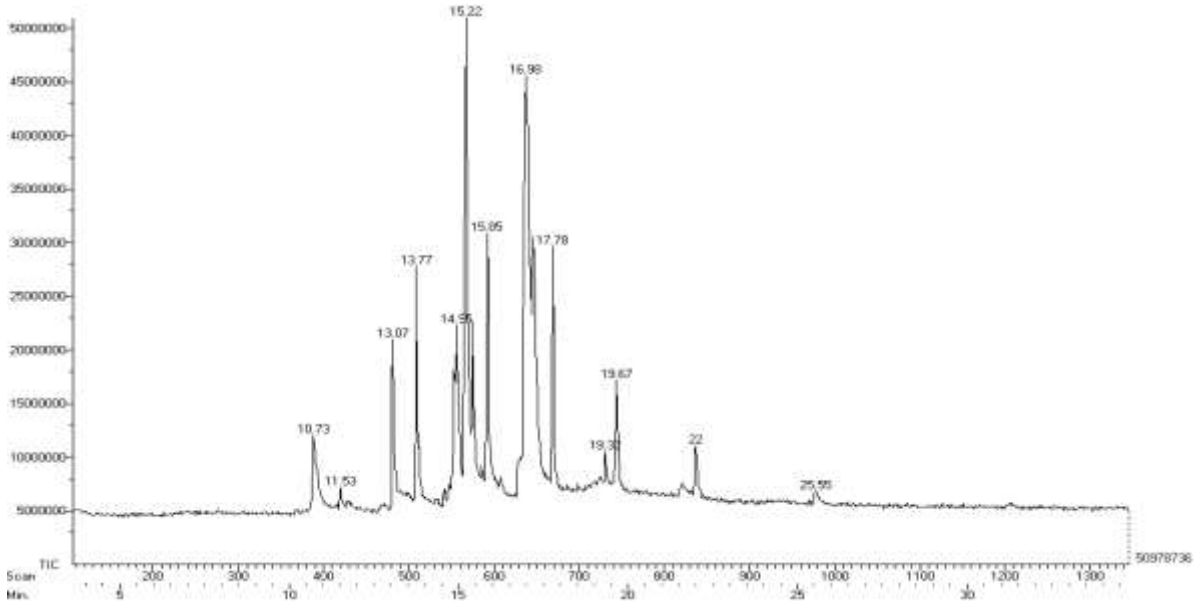

Fig. 2: GC-MS chromatogram of peel extract of *Hylocereus undatus*

The chemical compounds detected in pulp extract of *H. undatus* using GC-MS analysis were 1-hexadecene, methyl tetra-decanoate, E-7-octadecene, octadecene, methyl tetra-decanoate, E-7-octadecene, octadecene, 8-octadecanone, 19-norpregn-4-ene-3, 20-dione, octacosane, campesterol, amyrin and phenol 2,4-bis-(1,1-dimethylethyl). Chemical compounds detected in peel extract of *H. undatus* using GC-MS analysis were 1-cyclohexene, 1,3,3-trimethyl-2-(1-methylbut-1-en-3-on-1-yl), 1-pentadecene -2-methyl-, methyl tetradecanoate, 1-octadecene, pentadecanoic acid-13-methyl ester, 1-eicosene, 19-norpregn-4-ene-3,20-dione, tetracosane, octacosane, nonacosane, campesterol and amyrin.

Fruit peels usually contain a higher proportion of bioactive compounds than any other part of a fruit which contribute to the tremendous biological properties. However, the present study shows that the pulp extract had higher amounts of non-nutritive components along with excellent

therapeutic values. The differences observed in the present study could be mostly due to the presence of tiny numerous seeds embedded in pulp which was not removed at the time of extraction. Though differences were observed in the therapeutic values of pulp and peel extracts of *H. undatus*, GC-MS analysis indicated the presence of 19-norpregn-4-ene-3, 20-dione (steroidal progestin), campesterol (phytosterol) and amyrin (triterpene) in both the extracts. Generally, fruit peels are considered to be waste substrates. Instead of discarding the peels of *H. undatus*, the above mentioned compounds present in *H. undatus* peels can be extracted using conventional and non-conventional methods and productively be used in medical, pharmaceutical, food and cosmetic sectors. Similarly, the colour of white dragon fruit peel is either pink or red in colour and rich in betalains. Instead of using synthetic antioxidants and artificial food colorants, betalains present in dragon fruit peel can be extracted and utilized as natural antioxidants and food colorants.

Conclusion: This study showed that different non-nutritive compounds present in peel and pulp extracts of *Hylocereus undatus* have potential to decrease oxidative stress through their antioxidant, antidiabetic and anti-inflammatory activities. Though the pulp and peel extracts of *H. undatus* exhibited certain dominant functional properties, more experimental studies focusing on isolation of compounds using different analytical techniques and extraction methods are required for their successful use in food, cosmetics and pharmaceutical sectors. Further, *in vivo* animal and human studies are required to confirm the role of *H. undatus* in decreasing oxidative stress.

Conflict of interest: The authors declare no conflict of interest.

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