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Research article

Exploring the biological activities of the *Hylocereus polyrhizus* extract

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Key words: Pitaya, *Hylocereus polyrhizus*, antimicrobial, antioxidant, GC-MS, 5-cedranone, cell viability.

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Abstract

In the current study, the chemical constituents of the methanol extract of Pitaya (*Hylocereus polyrhizus*) were identified. The antimicrobial activity was evaluated via cup agar, and disk diffusion methods using five pathogenic bacterial & fungal strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *Fusarium oxysporum*. Also, the total antioxdant capacity (TAC) was evaluated via phosphomolybdenum method, and total phenolic content (TPC) was evaluated via Folin-Ciocalteu's assay. The GC-MS analysis revealed the presence of five identified compounds representing (91.15%) of the total composition *viz.*, 5-cedranone (73.05%), β-selinene (7.25%), eucalyptol (6.54%), and terpinolene (3.69%). The results showed that the methanol extract exhibited strong antimicrobial activity against the five strains expressed by inhibition zones as 29, 29, 29.5, 17.5, and 29.5 mm by cup agar method, and 9.5, 11, 10, 8, and 16.5 mm by disk diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *Fusarium oxysporum* respectively. Moreover the TAC value was 726.73 mg AAE/g dry extract, and the TPC was 432.88 mg gallic acid equivalent/g dry extract. In conclusion, the methanol extract of *Hylocereus polyrhizus* fruit showed strong antimicrobial activity, which may be return to its oxygenated terpenes like 5-cedranone, eucalyptol, and α-terpineol.

Introduction

Over the past few decades, the human health is under threat as many commonly used antibiotics have become less effective against certain illnesses [1]. Therefore, it was very necessary to examine newer drugs with higher efficiency. Natural compounds resulting from natural sources play a significant impact in the treatment and prevention of human diseases. In many developing countries, traditional medicine is one of the primary healthcare systems [2]. Herbs are widely exploited in the traditional medicine and their healing potentials are well demonstrated [3]. About 60% of recently developed drugs between 1981 and 2002 were based on natural products and they have been very effective, particularly in the field of infectious disease and cancer [4]. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world [4] Much work has been done on medicinal plants in India. Thus, the need to provide effective and safe compounds, which have a biological activity, redirected the attention to the extraction of bioactive compounds from plants [5, 6].

Pitaya (*Hylocereus* spp.) or dragon fruit originated principally from the tropical and subtropical forest regions of Latin Americas, including North, Central and South America [7, 8]. It is a perennial, epiphytic climbing cactus with triangular, fleshy, and jointed green stems [9]. The red pitaya is a rich source of nutrients and minerals *i.e.,* vitamin B1, vitamin B2, vitamin B3 and vitamin C, protein, fat, carbohydrate, crude fiber, flavonoid, thiamin, niacin, pyridoxine, kobalamin, phenolic, betacyanins, polyphenol, and carotene [10].

Red pitaya fruit is rich in phytoalbumins which exhibit high antioxidant activities [11]. Thus, the probiotic properties and high antioxidant uses of the red pitaya fruit have been reported [12, 13].

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Recently, antimicrobial activity of extract from dragon fruit (*Hylocereus polyrhizus*) against *Bacillus* spp., *Pseudomonas* spp., *Vibrio* spp., *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp., *Listeria* spp., *Salmone*lla spp., and *Aeromonas* spp. wad highlighted [14]. However, more screening and discussion still needed.

Therefore, this study aims to explore the biological functions (antimicrobial, antioxidant and anti-caners) of the extract with the identification of the major bioactive substances (chemical constituents) using the GC-MS analysis.

Experimental

Extract preparation

The stem were separated and washed with tap water and dried in the oven at 70 ℃ up to dryness. The dried and cleaned samples have been grounded into fine powder by electric mill. A certain weight of the dried powder was soaked in methanol 95% (with ratio of 1:10w/v) at room temperature for 24 hours in the dark. The liquid samples were filtered with Whatman filter paper (slow speed filtration). The supernatant was concentrated using rotary evaporator at 40℃, and the extract was stored at -20℃ [15].

Antimicrobial activity test by agar cup plate method

The sample was prepared by dissolving 0.01g in 2ml of methanol and 100µl (containing 500µg) was used in this test. The antimicrobial activity of the methanol extract from *Hylocereus polyrhizus* sample was investigated by the agar cup plate method. Five different test microbes namely: *Staphylococcus aureus* (G+ve), *Pseudomonas aeruginosa* (G-ve), *Candida albicans* (yeast), *Aspergillus niger* (fungus) and *Fusarium oxysporum* (fungus) were used. Nutrient agar plates were inoculated homogeneously with 1ml of 10^6-10^7 cells/ml in case of bacteria and yeast. A potato dextrose agar plates inoculated by the fungi were used to evaluate the antifungal activities. Then a hole was made in media by gel cutter (cork borer no. 4) in sterile condition. Then one drop of solidified agar was poured into the hole and left to solidify to make a base layer. After that definite amount of extract (0.1 ml) was poured into the hole. Then plates were reserved at 4°C for 2-4 hours to permit highest diffusion. The plates were then incubated at 37°C for 24 hours for bacteria and at 30°C for 48 hours in upright situation to make optimum growth of the organisms. The antimicrobial activities of the test extract were determined by measuring the diameter of clearance zone (inhibition) expressed in millimeter. The experiment was carried out more than once and mean of reading was recorded [16, 17]. The disk agar plate method has been constructed at the same time according to Bauer *et al.* [18].

Determination of total phenolic content (TPC)

The total phenolic content of plant extracts was determined using Folin - Ciocalteu's reagent according to the reported methods [19, 20].

Determination of total antioxidant capacity

The antioxidant activity of plant extracts was determined according to phosphomolybdenum method, using ascorbic acid as standard [21-23].

Cell Viability Assay using WST test

Metabolically active cell of *Staphylococcus aureus* were treated with different volumes of crude methanolic extract (15, 30, and 45µl/1 ml) for 3 hours. Consequently, the cell viability of was measured by using WST assay (WST-1: [2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium]) (Roche). 50 µl of the WST reagent was added to 1 ml of a bacterial cell suspension in PBS containing 10 g/l of glucose. The reagent was allowed to react for 30 min, before the absorbance was measured at 450 nm [24-25].

Cytotoxic activity test

The extracts showed high antioxidant activity were selected for further investigation of their cytotoxic activity. The cytotoxic activity of the selected extracts were tested against four cancer cell lines including; HELA (cervical), MCF-7 (breast), and HEPG2 (liver). All cell lines were obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). All cell lines were cultured in RPMI-1640 medium (Sigma Aldrich Chemical Co., St. Louis. Mo. U.S.A) supplemented with 10% FBS (Fetal bovine serum), penicillin (100 U/mL) and streptomycin (2 mg/mL) at 5%CO_2 in a 37°C incubator. The cells were plated in 96-well plate at a density of $3.0x10^3$ in 150 µL of medium per well. Tested extracts dissolved in DMSO were added to the wells in triplicates with concentrations of 0, 5, 12.5, 25 and 50 µg/mL for 48hrs. The cytotoxic activity was determined using Sulphorhodamine-B (SRB) assay following the method reported by Vichai and Kirtikara [26]. The IC values (the concentrations of extract required to produce 50%inhibition of cell growth) were also calculated.

GC/MS Analysis

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS and TGSMS Fused Silica Capillary Column (30m, 0.251 mm, 0.1 mm Film thickness), National Research Center, Giza, Egypt. For GC/MS detection, an electron ionization system with ionization energy for 70 ev was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 40°C (hold 3 min) to 280°C was a final temperature at an increasing rate of 5°C /min (hold 5 min). The identified components were investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative

retention time and mass spectra with those of the NIST, WILLY Library data of the GC/MS system [27-28].

Results and Discussion

Antimicrobial activity

The antimicrobial activity was evaluated via cup agar (100 microliter per cup), and disk diffusion (20 µl/disk) methods using five pathogenic bacterial & fungal strains *e.g. Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *Fusarium oxysporum*. The results in Tables (1, 2) and Figure 1 showed that the 95% methanol extract exhibited a strong antimicrobial activity against the five strains expressed by inhibition zones as 29, 29, 29.5, 17.5, and 29.5 mm by cup agar method, and 9.5, 11, 10, 8, and 16.5 mm by disk diffusion method against *S. aureus*, *P. aeruginosa*, *C. albicans*, *A. niger*, and *F. oxysporum* respectively. The extract of *Hylocereus polyrhizus* fruit showed strong antimicrobial activity, which may be return to the synergistic action (Co-activity) of its oxygenated terpenes like 5-cedranone, eucalyptol, and α-terpineol [29].

Cell Viability Assay using WST test

After 3 hours of treatments using different concentrations (15, 30, and 45µl/1 ml), the proliferation of cell suspension was monitored. The results showed that a clear inhibition effect on the cell proliferation. Therefore, the decrease in the bacterial cell activity was strongly dependant on the concentration of the crude extract, whereas the more viable the bacterial cells were (low concentration of the crude extract), the higher metabolic activity, as demonstrated in Figure 2.

Table 1. The antimicrobial activity of the 95% methanol extract of *H. polyrhizus* using cup agar method in 100 µl/cup

Clear zone (ϕmm)				
S. aureus	***P. aeruginosa***	***C. albicans***	***A. niger***	***F. oxysporum***
29 ± 1.41	29±2.82	29.5± 0.70	17.5± 0.70	29.5± 0.70

Table 2. The antimicrobial activity of the 95% methanol extract of *H. polyrhizus* using disk agar method in 20microliter per disk.

Clear zone (ϕmm)				
S. aureus	***P. aeruginosa***	***C. albicans***	***A. niger***	***F. oxysporum***
9.5± 0.70	11± 1.41	10± 0.0	8± 0.0	16.5± 2.12

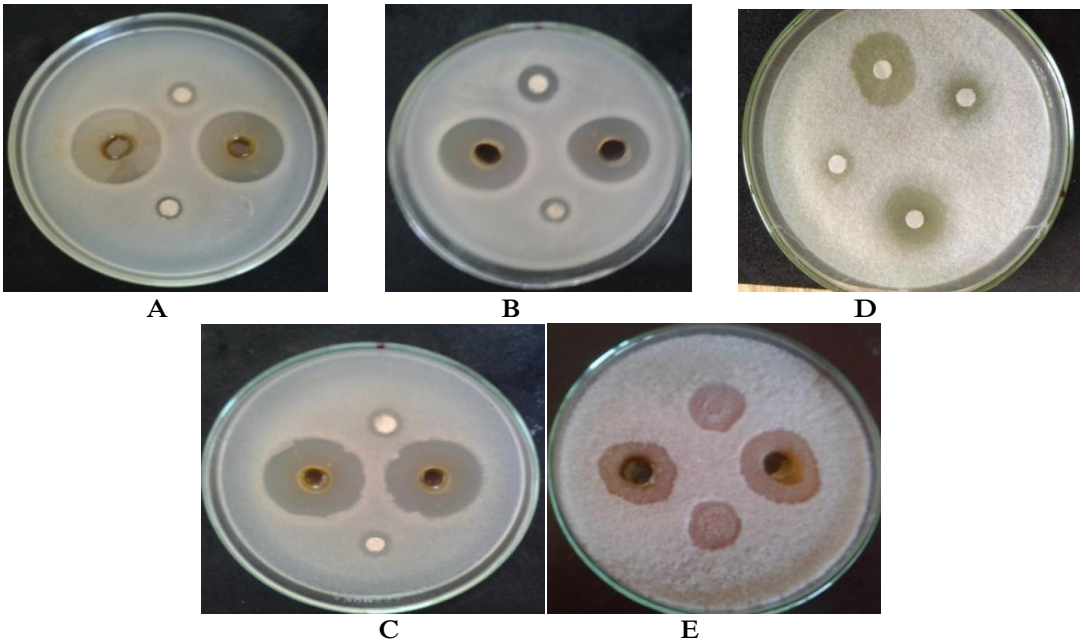


Figure 1. The antimicrobial activity of *Hylocereus polyrhizus* extract using cup and disc agar plate methods against *Staphylococcus auerus* (A), *Pseudomonas aeruginosa* (B), *Candida albicans* (C), *Aspergillus niger* (D) and *Fusarium oxysporum* (E).

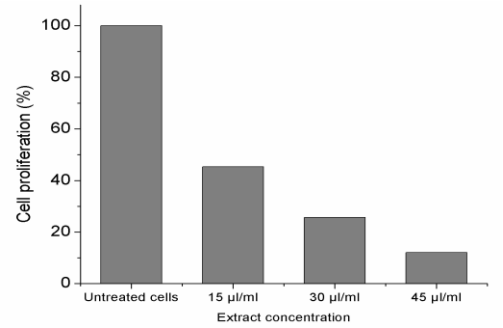
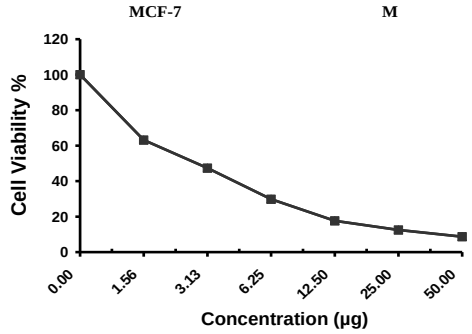


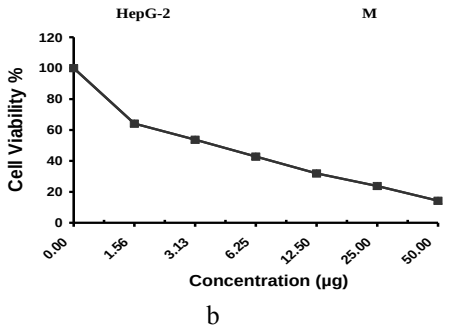
Figure 2. The viability of *S. aureus* cells using different concentrations of *H. polyrhizus* 95%methanol extract

Cytotoxicity activity

The methanol extract from *H. polyrhizuz* exhibited in vitro good cytotoxic activity against breast (MCF-7) and liver (HepG-2) carcinoma (Figure 3. a, and b). This extract has IC_{50} of 2.8 and 4.2µg for breast and liver cell lines, respectively. Two extracts from two different species *of Hylocereus*, namely: *Hylocereus polyrhizuz* and *Hylocereus undatus*, were tested for their cytotoxic against human prostate (PC3), human breast (Bcap-37), and human gastric (MGC-803) cell lines [30]. The extract of *H. polyrhizuz* showed IC_{50} of 0.61, 0.45 and 0.43 µg for PC3, Bcap-37 and MGC-803, respectively. But the *H. undatus* extract exhibited IC_{50} of 0.64, 0.47 and 0.73 µg for PC3, Bcap-37 and MGC-803, respectively.



a



b

Figure 3. Effects of the 95% methanol extracted from *Hylocereus polyrhizus* on MCF-7 (Breast) and HEPG2 (Liver carcinoma) cell lines viability.

GC-MS analysis

The GC-MS analysis of the 95 % methanol extract of *H. polyrhizus* revealed the presence six compounds (Figure 4 and Table 3), from which five components were identified represent (91.15%) of the total oil composition. 5-cedranone (73.05%), β-selinene (7.25%), eucalyptol (6.54%), and terpinolene (3.69%). Luo *et al.,* [30] reported that the main components of *H. polyrhizus* growing in China were β-amyrin (15.87%), α-amyrin (13.90%), octacosane (12.2%), γ-sitosterol (9.35%), octadecane (6.27%), 1-tetracosanol (5.19%), stigmast-4-en-3-one (4.65%), and campesterol (4.16%) [30].

Total Phenolic content

The total phenolic content of the methanol extract of *H. polyrhizus* was 432.88 mg gallic acid equivalent/g dry extract. Chemah *et al.,* [31] reported that the ethanolic extract of *H. polyrhizus* seeds showed phenolic content (43.9 mg gallic acid equivalent/100g dry weight [31]. Also, it was found that red pitaya showed high phenolic content (16.70 GAE/100g) [32].

Antioxidant activity

The results revealed that the total antioxidant capacity (TAC) of the 95% methanol extract was 726.73 mg AAE/g dry extract. The antioxidant activity of *H. polyrhizus* is well known [30-32]. High phenolic content were usually correlated with high radical scavenging activity [33]. Choo and Yong [34], reported that *H. polyrhizus* have great antioxidant activity due to its high phenolic content (15.92 mg gallic acid/g) [34].

Conclusion

In the current study, exploring the biological activities of the *Hylocereus polyrhizus* extract was performed, followed by components identification using the GC-MS analysis. The methanolic extract showed very interesting antimicrobial properties against several pathogenic strains. The Cells viability confirmed the sensitivity of the pathogens to the low concentration of the extract. Furthermore, positive effects was founds as antitumor's when different cancer cell-lines were examined. Frequently, The Total Phenolic content was identified and thus antioxidant features were found.

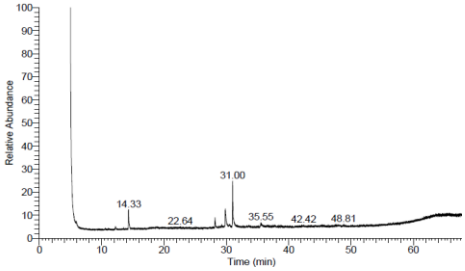
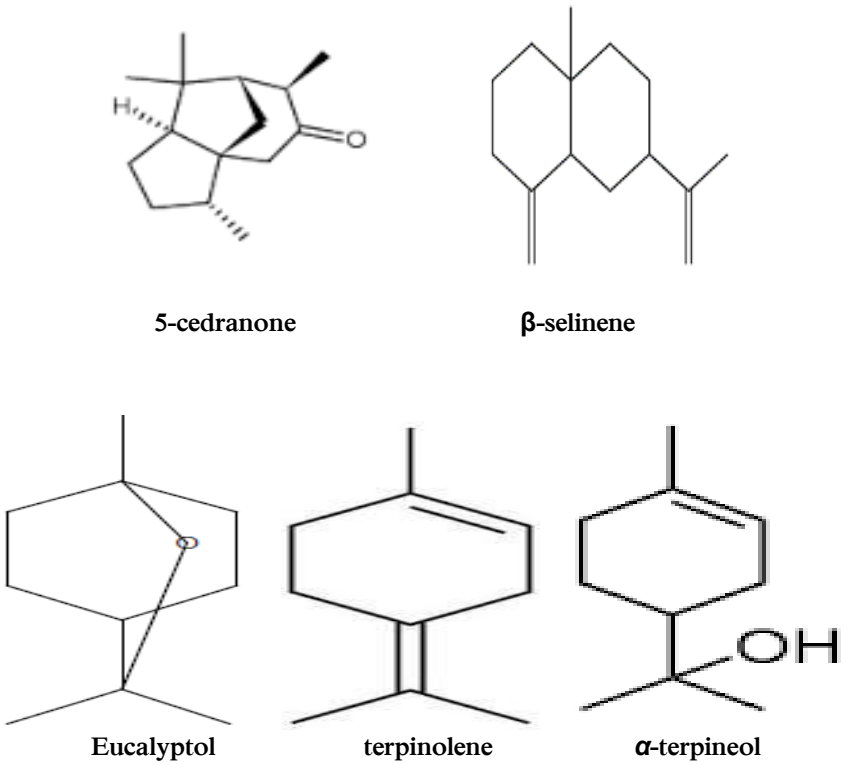


Figure 4a. Gas chromatography-mass spectrometry (GC/MS) chromatogram of the 95 % methanol extract of *H. polyrhizus.*



5-cedranone β-selinene

Eucalyptol terpinolene α-terpineol

Figure 4b. Chemical structure of the identified constituents in the 95% methanol extract of *H. polyrhizus.*

Table 3. Chemical compositions of 95% methanol extract of *H. polyrhizus.*

No	R.T.	Area %	M.W.	M.F.	Main Fragments	Identified compounds
1	12.21	3.69	136	$C_{10}H_{16}$	(41, 51, 67, 79, 93, 105, 121, 136)	Terpinolene
2	14.32	6.54	154	$C_{10}H_{18}O$	(41, 55, 67, 71, 81, 108, 139, 154)	Eucalyptol
3	22.47	0.62	154	$C_{10}H_{18}O$	(43, 55, 59, 67, 81, 93, 107, 121, 136)	α-terpineol
4	28.21	7.25	204	$C_{15}H_{24}$	(41, 53, 67, 79, 93, 105, 119, 133, 161, 189, 204)	β-selinene
5	29.83	12.06	----	----	----	Unknown
6	31.0	73.05	220	$C_{15}H_{24}O$	(41, 55, 67, 95, 123, 149, 163, 191, 205, 220)	5-cedranone
T%		91.15				

[1]Compounds identified via comparison its mass spectrum with NIST library, Adams, 2001 and literature.
M.F.: Molecular formula; M.W.: Molecular weight; R.T.: Retention time

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