Nutritional Composition Assessment and Antimicrobial Activity of Catostylus perezi, Jellyfish Blooms along the Coast of Pakistan: An Awareness to Avoid Food Neophobia in Pakistan

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ABSTRACT

This study highlighted the nutritional importance of *Catostylus perezi* (an edible jellyfish) in Pakistan; a society where a large proportion of the population suffers from malnutrition, while *C. perezi*, a blessing of the sea, is wasted or exported. In the present study, the amino acid and fatty acid profiles of the oral arms and umbrella of *C. perezi* were determined by HPLC. The total amino acid concentration (ΣAA) in the oral arms was 151.19 mg/100g, while in the umbrella it was 100.17 mg/100g. The ratio of total essential amino acids to total non-essential amino acids

(TEAA/TNEAA) was 0.72 in the oral arms, while it was 0.70 in the umbrella. Higher amount of ω-3 with lower ratio of ω-6/ω-3 in oral arms (0.52), rather umbrella (ω-6/ω-3 ratio; 0.62). The antimicrobial activity, MIC, MBC and MFC of the whole body of the edible jellyfish were determined. On the basis of polarity, different solvents were used, e.g. water, methanol, dichloromethane, chloroform and n-hexane. Among all the extracts, the water extract gave the highest ZOI against *C. xerosis* (29 mm), while the n-hexane extract gave the lowest ZOI against *S. aureus* (MRSA) ATCC 33591 (8.20 mm). The water extract of *C. perezi* had high potential against *C. xerosis* with the highest AMI and PAI (1.53 and 153, respectively), while the same extract had the highest TAI against *E. coli* (81.43 mL/g). For fungi/yeast, the methanolic extract had the highest ZOI (29.70 mm) against *S. cerevisiae* and the lowest MIC/MFC (2.40 µg /mL) against the same pathogen. The n-Hexane extract gave the lowest ZOI (11.10 mm) against *P. variotii* and the highest MIC/MFC (31.60 µg /mL) against *Penicillium* sp. Atomic force microscopy (AFM) was used to analyze the disintegrating effect of the extracts (with the highest antimicrobial effect) on the cells of selected Gram-positive, Gram-negative and yeast species. The amino acid and fatty acid profiles and antimicrobial assessment showed that *C. perezi* has great nutritional importance, so the use of *C. perezi* as food is highly recommended for the Pakistani community.

Supplementary Material

1. Experimental Section

1.1. Sample collection, Identification and Preparation

Jellyfish (*C. perzi*) samples were collected in May 2022 from Sonmiani Bay on the Arabian Sea in Lasbela district, Balochistan, Pakistan (25° 30' 55.76" N'; 66° 32' 29.41" E). The identification of jellyfish species was based on characters of the species described by Gul and Morandini, (2013). The specimens are available at the Centre of Excellence in Marine Biology, University of Karachi, Karachi Pakistan (collection specimens of the third co-author) under voucher number CEMB 127875. Freshly caught jellyfish samples were classified into two categories: Whole body and dissected into umbrella and oral arms. Samples were stored in an ice-box and transported to the laboratory where they were immediately dried using the Edwards Supermodulyo freeze dryer at -40 °C for 48-72 h until completely dried. Samples were stored at -20 °C until use (Chan et al., 2021a, 2021b).

1.2. Amino acids analysis

Freeze-dried *C. perzi* samples were hydrolyzed according to the method described in (AOAC, 2015). 25 mg of sample was taken into screw-capped digestion tubes. 2 mL of 6 N HCl was added and incubated at 110 °C for 24 h under vacuum. The hydrolyzed sample was transferred to a rotary evaporator flask and evaporated to dryness under vacuum at 70 °C. The final volume was made up to 25 mL with deionized water. The sample was filtered through a 0.22-µm syringe filter and diluted with buffer solution. 20 µL of the diluted sample was injected into the HPLC-based amino acid analyzer (Shimadzu Amino Acid Analyzer with Shim-Pack Amino-Na column, 4.6 mm, I.D x100 mm). A post-column derivatization reaction of the eluted amino acids with o-phthalic dihyde (OPA) was used to detect the peaks with a fluorescence detector. The detector wavelength was set at 350 nm for excitation and 450 nm for emission.

1.3. Fat extraction

100 g of freeze-dried samples were ground into fine powder and by the solvent extraction method (chloroform and methanol) used in our previous study, the fat was extracted (Ullah et al., 2023a).

1.4. Fatty acid analysis

Fatty acids were hydrolyzed and derivatized from total lipids according to the procedure used in our previous study (Ullah et al., 2023a). Gas chromatography was performed using a GC-2010, Shimadzu Corporation 07947, equipped with FID detector, split injector and SP-2560 silica fused capillary column (100 m x 0.25 mm x 0. 2 µm, Supelco) with the following operating program: Injection volume 1 µL with temperature 250 °C, detector temperature 260 °C, column temperature 140 °C for 5 minutes and then ramped to 240 °C at 4 °C per minute, remained stable for 15 minutes; helium was used as carrier gas with a flow rate of 1.12 mL/min and a linear velocity of 20 cm/s; split ratio 1:100. Results were expressed as relative percentages of FID response area.

1.5. Preparation of the samples for antimicrobial activity

The freeze-dried jellyfish sample (whole body) was soaked in various solvents (water, methanol, dichloromethane, chloroform, and n-hexane) and stored at 4 °C for 72 h. After centrifugation at 9000 × g for 10 min, the supernatants were freeze-dried to obtain a powder form and used for antimicrobial activity (Chan et al., 2021a, 2021b).

1.6. Bacterial and fungal strains

Cultures were indigenously obtained from clinical and environmental sources, stored at 4 °C onto tryptic soy agar, Czapek's agar and yeast extract agar (Oxoid, UK). 10 Gram-positive pathogens, including; *Bacillus cereus*, *Bacillus subtilis* (Hay bacillus), *Corynebacterium xerosis*, *Enterococcus faecalis*, VRE *Enterococcus faecalis* (ATCC 51299), *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus aureus* (MRSA) ATCC 33591, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, were used. 10 Gram-negative bacteria; *Escherichia coli*, *Enterobacter* species, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Shigella* species, *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella paratyphi* B and *Vibrio* species. Twelve fungal species, including eight saprophytes; *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus terricola*, *Penicillium* species, *Paecilomyces variotii*, *Chrysosporium* species, and *Fusarium oxysporum*, two dermatophytes; *Trichophyton mentagrophytes* and *Microsporum gypseum* and two yeast species were used in the study (*Candida albicans* and *Saccharomyces cerevisiae*) (Chan et al., 2021a, 2021b).

1.7. Antimicrobial Assay

For antimicrobial activities, the agar well diffusion method was used (Chan et al., 2021a; Chan et al., 2021b; Naveed et al., 2023). Standard solutions of ciprofloxacin (10 µg/mL) and miconazole (10 µg/mL) were used for bacterial and fungal species as positive control. Dimethylsulfoxide (5% v/v) was used as negative control.

Both bacterial and yeast cultures were incubated overnight at 37 °C in Sabouraud dextrose broth (Oxoid, U.K.), centrifuged at 9000 x g, and the supernatant removed. A cell suspension, equivalent to 0.5 McFarland standard (1.5×10^8 CFU/mL) was used. Confluent lawns were made on fresh MHA and Sabouraud dextrose agar (SDA) plates. The wells were dug (6 mm) , solutions of 20 µL extracts dispensed into them and allowed to diffuse into media for 15-20 min. Same methodology was used for the fungal culture only incubation period was different (at 25 °C for 5 days) (Chan et al., 2021a; Chan et al., 2021b).

1.8. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC)

For the determination of MIC and MBC, we used the dilution method described in our previous study (Naveed et al., 2023).

1.9. Biological indices

Different biological indices were used to evaluate the antimicrobial potential of extracts of *C. perezi* (Chan et al., 2021a; Chan et al., 2021b). These indices were; antimicrobial index (AMI), percentage activity index (PAI) and total activity index (TAI).

$$\mathrm{AMI} = \frac{\text{ZOI of the Extract}}{\text{ZOI of Antibiotic}} \qquad \text{(I)}$$

$$\mathrm{PAI} = \frac{\text{ZOI of the Extract}}{\text{ZOI of Antibiotic}} \times 100 \qquad \text{(II)}$$

$$\mathrm{TAI} = \frac{\text{Extract per gram of dried tissue}}{\text{MIC of the same extract}} \qquad \text{(III)}$$

1.10. Atomic Force Microscopy (AFM) Analysis

C. xerosis, *E. coli* and *S. cerevisiae* were used for AFM analysis. The effective concentrations were the same as those used for MIC/MBC. The same method used in our previous studies was applied (Naveed et al., 2023; Ullah et al., 2023b).

1.11. Statistical analysis

Data obtained from the analysis was expressed as mean ± S.D (n = 9). The results were analyzed using Microsoft Excel (ver. 10) and Multivariate Analysis of Variance (MANOVA) followed by Post Hoc Tukey test using SPSS ver. 23.0 (Chicago, IL, USA). All data had been tested for homogeneity in variation before significant analysis. Values of $P < .05$ were considered statistically significant.

Fig S1. The images of *C. xerosis* by atomic force microscopy (AFM). **A;** the cells of *C. xerosis* before the treatment of water soluble extract of *C. perezi* and **B;** representing the cells of *C. xerosis* after the treatment of water soluble extract of *C. perezi*

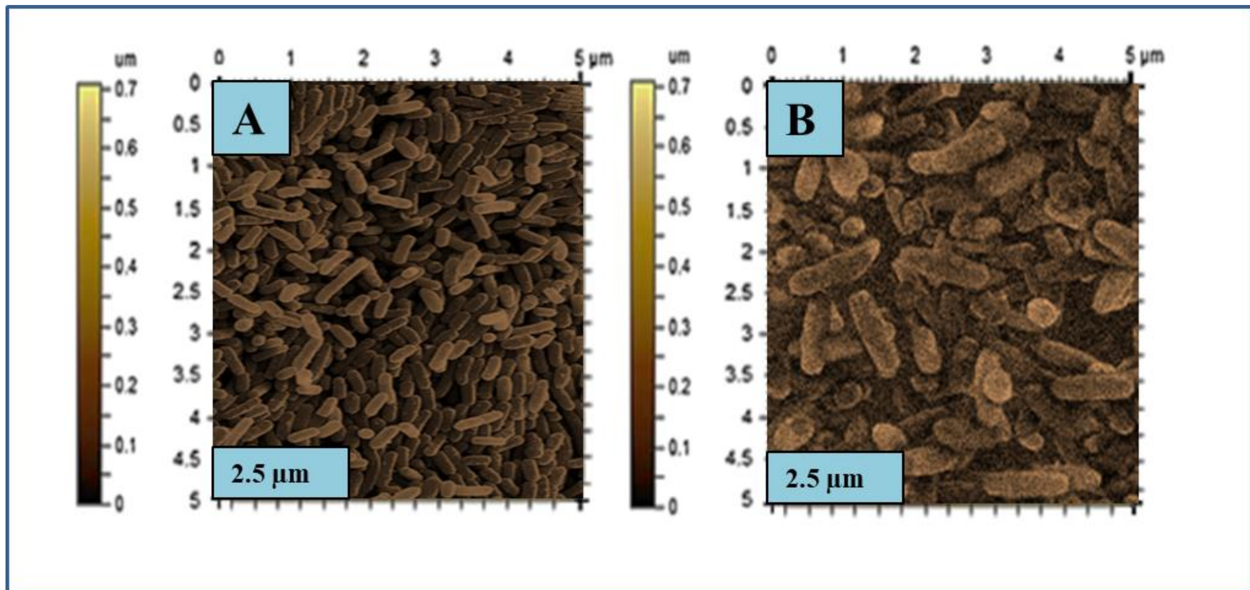

Fig S2. The images of *E. coli* by atomic force microscopy (AFM). **A;** the cells of *E. coli* before the treatment of water soluble extract of *C. perezi* and **B;** representing the cells of *E. coli* after the treatment of water soluble extract of *C. perezi*

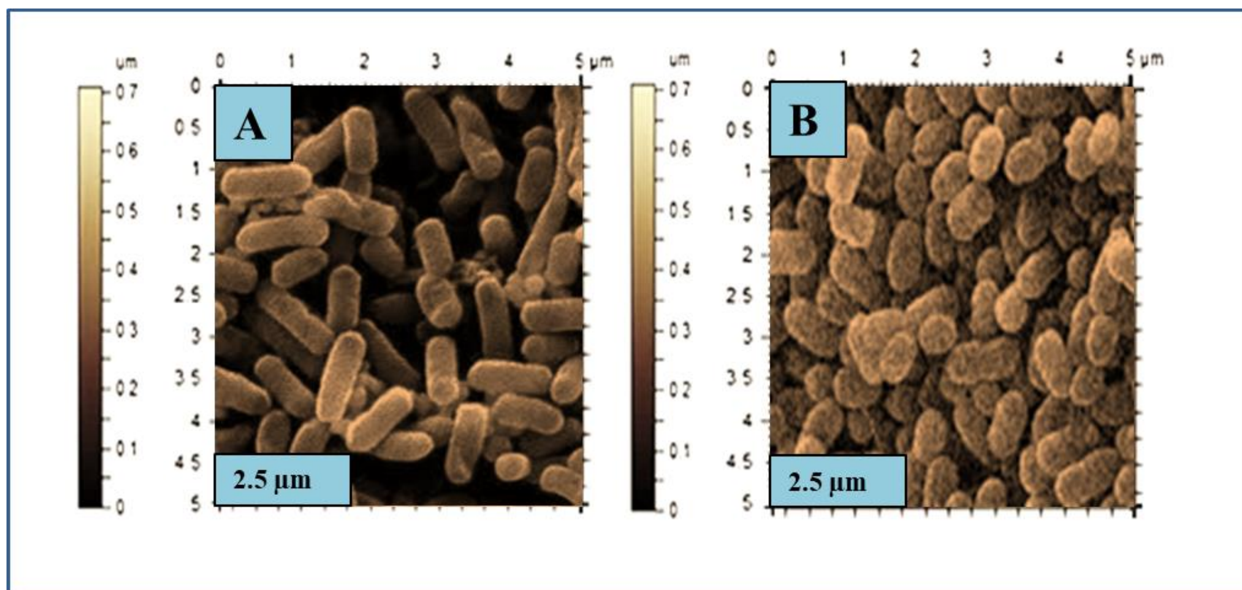

Fig S3. The images of *S. cerevisiae* by atomic force microscopy (AFM). **A;** the cells of *S. cerevisiae* before the treatment of water soluble extract of *C. perezi* and **B;** representing the cells of *S. cerevisiae* after the treatment of water soluble extract of *C. perezi*

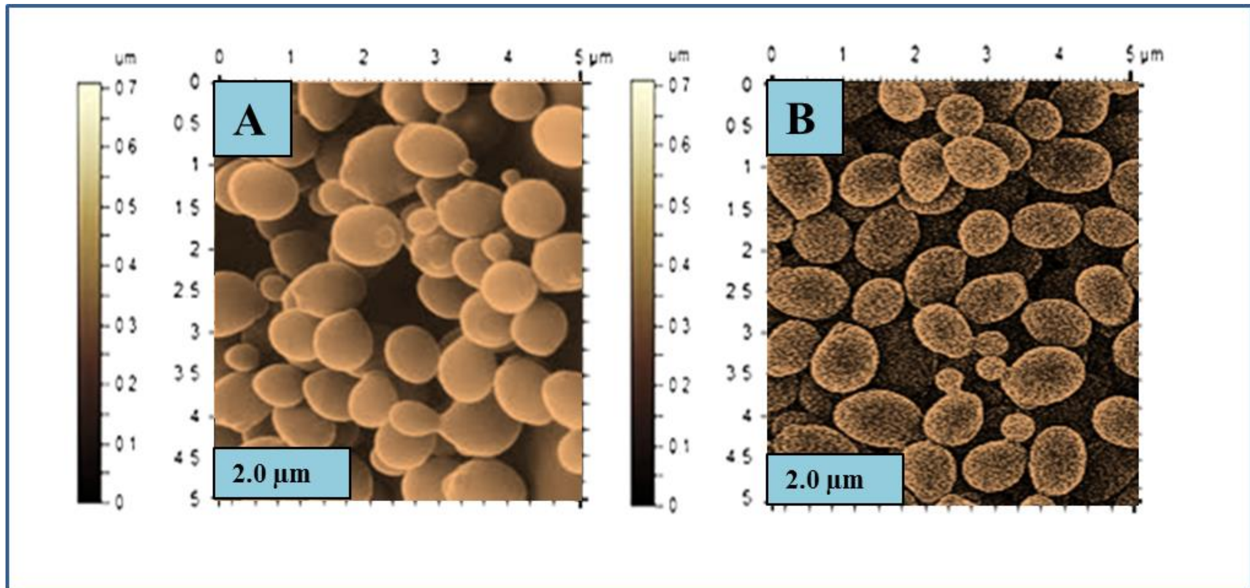

Table S1. Amino acids profile of umbrella and oral arms of *C. perezi*

Amino acids	Umbrella (mg/100g)±SD	Oral Arms (mg/100g)±SD
Aspartic acid	9.25±0.39[b]	11.80±0.45[a]
Threonine	4.50±0.20[b]	7.75±0.27[a]
Serine	3.25±0.20[b]	6.14±0.29[a]
Glutamic acid	9.01±0.33[b]	12.92±0.40[a]
Proline	4.05±0.27[b]	6.8±0.31[a]
Glycine	20.04±0.88[b]	25.44±0.94[a]
Alanine	3.47±0.25[b]	5.62±0.21[a]
Cystein	2.01±0.17[b]	5.11±0.19[a]
Valine	2.27±0.24[b]	4.41±0.20[a]
Metheionine	3.10±0.18[b]	5.16±0.20[a]
Isoleucine	4.16±0.29[b]	6.27±0.32[a]
Leucine	3.20±0.30[b]	5.47±0.41[a]
Tyrosine	4.77±0.39[b]	8.80±0.35[a]
Phenylalanine	7.51±0.49[b]	11.85±0.55[a]
Histidine	0.99±0.02[b]	1.50±0.04[a]
Tryptophan	1.17±0.04[b]	2.26±0.03[a]
Lysine	10.68±0.45[b]	15.71±0.71[a]
Arginine	6.74±0.41[b]	8.18±0.56[a]
ΣAA	100.17±5.50[b]	151.19±6.43[a]
ΣEAA	37.58±2.21[b]	60.38±2.73[a]
ΣCAA	49.87±2.65[b]	73.39±3.04[a]
ΣAAA	13.45±0.91[b]	22.91±0.94[a]
TEAA/TAA	0.37	0.41
TEAA/TNEAA	0.7	0.72

ΣAA; sum of total amino acids, ΣEAA; sum of essential amino acids, ΣCAA; sum of conditionally essential amino acids, ΣAAA; sum of aromatic amino acids, TEAA/TAA; ratio of total essential amino acids and total amino acids, TEAA/TNEAA; ratio of total essential amino acids and total non-essential amino acid

Data (means ± SD, n = 9) followed by different letters (superscripts) indicate significant differences among variables (a = $P < 0.01$; b = $P < 0.05$)

Table S2. Fatty acid composition and total lipids of umbrella and oral arms of *C. perezi*

Fatty Acid (FA)	Umbrella	Oral Arms
Saturated FA (SFA)	Percentage±SD	Percentage±SD
Lauric acid C12:0	6.45±0.20[a]	6.07±0.24[b]
C13:0	1.31±0.08[a]	1.28±0.07[a]
Myristic acid C14:0	4.19±0.34[a]	4.11±0.36[a]
C15:0	2.91±0.23[a]	2.70±0.20[b]
Palmitic acid C16:0	21.41±1.23[a]	20.04±1.32[b]
C17:0	6.11±0.18[a]	6.10±0.39[a]
Stearic acid C18:0	10.95±0.68[a]	10.08±0.56[b]

Arachidic acid C20:0	1.09±0.11^{a}	1.04±0.13^{a}
C22:00	0.41±0.05^{a}	0.39±0.09^{a}
Total SFA	54.83	51.8
Monounsaturated FA		
C14:1	0.71±0.08^{a}	0.84±0.08^{a}
Palmitoleic acid C16:1 (ω7)	2.05±0.10^{a}	2.20±0.12^{a}
Palmitoleic acid C16:1 (ω9)	3.59±0.15^{b}	3.71±0.16^{a}
C17:1	0.89±0.09^{a}	0.98±0.08^{a}
Oleic acid C18:1 cis-9 (ω9)	4.57±0.25^{b}	4.75±0.22^{a}
Isoleic acid C18:1 trans-10	0.61±0.07^{a}	0.74±0.09^{a}
C22:1	1.89±0.14^{b}	2.03±0.13^{a}
Total MUFA	14.31	15.24
Polyunsaturated FA		
Linoleic acid C18:2 cis-9,12 (ω6)	1.19±0.08^{b}	1.37±0.10^{a}
Linolenic acid C18:3 cis-9,12,15 (ω3)	1.52±0.14^{b}	1.95±0.22^{a}
Stearidonic acid C18:4 (ω3)	4.73±0.35^{b}	5.12±0.25^{a}
C20:2	0.43±0.05^{a}	0.51±0.08^{a}
Arachidonic acid C20:4 (ω6)	8.61 ± 0.69^{a}	7.79 ± 0.53^{b}
Eicosapentaenoic acid C20:5 (ω3)	2.39±0.17^{a}	2.71±0.26^{a}
Docosatetraenoic acid C22:4 (ω6)	1.87±0.10^{a}	1.91±0.16^{a}
Docosapentaenoic acid C22:5 (ω3)	3.11±0.11^{b}	3.59±0.29^{a}
Docosahexaenoic acid C22:6 (ω3)	7.01±0.22^{b}	8.01±0.53^{a}
Total PUFA	30.86	32.96
Total fatty acids		
Fatty acids ∑ω6	11.67	11.07
Fatty acids ∑ω3	18.76	21.38
Ratio ω6/ω3	0.62	0.52
Total Lipids (g/100 g dry weight)	1.9	3.5

SFA; saturated fatty acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids, Σω6; total ω6 fatty acids, Σω3; total ω3 fatty acids

Data (means ± SD, n = 9) followed by different letters (superscripts) indicate significant differences among variables (a = $P < 0.01$; b = $P < 0.05$)

Table S3. Antibacterial activity indicating zone of inhibition (ZOI), minimum inhibitory and bactericidal concentrations (MICs and MBCs) of different solvents extracts of whole body of *C. perezi*

	ZOI (mm)±SD					MIC & MBC (µg /mL)±SD				
Organisms	**Water**	**Methanol**	**Dichloromethane**	**Chloroform**	**n-Hexane**	**Water**	**Methanol**	**Dichloromethane**	**Chloroform**	**n-Hexane**
S. aureus	15.1±0.74^{a}	14.4±0.65^{b}	12.4±0.42^{c}	11.7±0.57^{d}	09.1±0.74^{e}	24.50±0.79^{e}	27.60±0.42^{d}	29.10±1.48^{c}	31.30±0.84^{b}	39.00±1.58^{a}
S. saprophyticus	18.0±0.35^{a}	17.1±0.79^{b}	15.2±0.57^{c}	13.7±0.57^{d}	11.8±0.57^{e}	21.00±0.79^{e}	24.00±0.42^{d}	25.60±0.65^{c}	29.00±0.79^{b}	31.80±2.17^{a}
S. epidermidis	21.1±0.74^{a}	19.8±0.57^{b}	18.1±0.74^{c}	17.4±0.42^{d}	13.3±0.84^{e}	19.60±0.65^{e}	22.30±0.57^{d}	23.30±0.84^{c}	25.30±0.84^{b}	27.00±1.58^{a}
S. aureus (MRSA) ATCC 33591	14.9±0.65^{a}	13.7±0.65^{b}	13.1±0.42^{b}	11.6±0.42^{d}	08.2±0.57^{e}	26.60±0.65^{e}	28.90±0.57^{d}	30.00±0.79^{c}	32.20±0.76^{b}	41.40±1.14^{a}
B. subtilis	28.9±0.42^{a}	27.6±0.79^{b}	25.6±0.65^{c}	25.5±0.50^{cd}	20.6±0.65^{e}	04.00±0.79^{e}	08.00±0.42^{d}	11.30±1.20^{c}	13.00±0.79^{b}	17.00±1.58^{a}
B. cereus	27.1±0.74^{a}	25.6±0.57^{b}	25.7±0.67^{b}	23.6±0.42^{d}	20.5± 1.00^{e}	04.60±0.42^{e}	06.70±0.42^{d}	07.70±0.84^{c}	08.80±0.57^{b}	17.00±1.00^{a}
M. luteus	25.1±0.74^{a}	23.2±0.65^{b}	23.1±0.74^{b}	21.4±0.42^{d}	17.9±0.65^{e}	06.20±0.57^{e}	08.90±0.76^{d}	12.50±1.12^{c}	14.28±0.56^{b}	21.40±1.14^{a}
C. xerosis	29.0±0.61^{a}	28.1±0.42^{b}	27.3±0.84^{c}	26.4±0.55^{d}	21.9±0.55^{e}	03.50±0.50^{e}	05.40±0.42^{d}	09.70±0.57^{c}	10.80±0.57^{b}	16.80±0.84^{a}
VRE E. faecalis (ATCC 51299)	15.1±0.42^{a}	13.9±0.79^{b}	11.7±0.67^{c}	10.7±0.57^{d}	08.3±0.57^{e}	22.60±0.65^{e}	27.50±0.65^{d}	30.70±1.20^{c}	32.60±0.42^{b}	41.40±1.82^{a}
E. faecalis	16.3±0.84^{a}	14.1±0.79^{b}	12.2±0.76^{c}	12.4±0.42^{c}	09.6±0.42^{e}	18.00±0.79^{e}	29.00±0.42^{d}	30.30±0.84^{c}	32.40±0.96^{b}	34.40±0.89^{a}
Vibrio sp.	14.3±0.57^{a}	12.7±0.57^{b}	10.9±0.65^{c}	10.7±0.67^{d}	10.0±0.50^{e}	24.10±0.74^{e}	30.80±0.57^{d}	35.50±1.66^{c}	38.50±1.00^{b}	31.40±1.14^{a}
E. coli	24.3±0.84^{a}	24.2±0.42^{b}	23.6±0.42^{c}	22.4±0.42^{d}	20.1±0.74^{e}	02.10±0.42^{e}	08.90±0.76^{d}	12.90±0.89^{c}	14.50±0.79^{b}	18.20±0.84^{a}
K. pneumoniae	19.9±0.65^{a}	18. 0±0.76^{b}	16.9±0.42^{c}	15.8±0.57^{d}	15.9±0.42^{e}	08.40±0.65^{e}	14.70±0.79^{d}	15.80±0.57^{c}	17.40±0.96^{b}	21.60±1.14^{a}
Shigella sp.	26.1±0.74^{a}	24.1±0.42^{b}	23.1±0.74^{c}	22.1±0.42^{d}	18.7±0.67^{e}	05.90±0.65^{e}	09.60±0.74^{d}	13.20±1.04^{c}	13.20±0.76^{b}	19.00±1.58^{a}
P. mirabilis	19.3±0.84^{a}	17.9±0.82^{b}	16.9±0.55^{c}	16.0±0.50^{d}	14.2±0.76^{e}	10.50±0.79^{e}	15.10±0.65^{d}	15.20±0.57^{c}	17.20±0.76^{b}	18.40±1.34^{a}
P. aeruginosa	21.3±0.84^{a}	20.2±0.57^{b}	18.1±0.74^{c}	17.3±0.57^{d}	17.1±0.74^{e}	11.50±0.50^{e}	15.80±0.76^{d}	17.00±0.79^{c}	19.00±0.79^{b}	24.30±0.97^{a}
Enterobacter sp.	20.0±0.79^{a}	18.7±0.57^{b}	16.8±0.63^{c}	15.7±0.57^{d}	16.5±0.61^{e}	14.10±0.74^{e}	17.20±0.57^{d}	18.80±0.57^{c}	20.10±0.42^{b}	21.94±0.85^{a}
S. typhi	18.3±0.97^{a}	16.2±0.57^{b}	14.3±0.57^{c}	13.2±0.57^{d}	11.8±0.57^{e}	16.30±0.84^{e}	23.80±0.76^{d}	26.50±1.00^{c}	29.30±0.57^{b}	33.00±1.37^{a}
S. paratyphi A	15.5±0.79^{a}	13.8±0.79^{b}	11.9±0.74^{c}	11.8±0.42^{c}	09.6±0.42^{e}	17.50±1.00^{e}	27.00±0.57^{d}	29.60±0.65^{c}	31.40±0.96^{b}	37.40±0.96^{a}
S. paratyphi B	15.8±1.04^{a}	13.9±0.50^{b}	12.6±0.42^{c}	11.8±0.27^{d}	08.1±0.42^{e}	20.00±0.79^{e}	28.50±0.96^{d}	32.70±1.2^{c}	35.80±1.30^{b}	39.10±0.74^{a}

Data (±SD, n=9) followed by different letters (superscripts) indicate significant differences among variables ($a = p < .001$; $b = p < .01$; c, d,$e = p < .05$)

Table S4. Antimicrobial index (AMI), percentage activity index (PAI) and total activity index (TAI) of different solvents extracts of whole body of *C. perezi*, calculated for Gram-positive and negative pathogens

		Water			Methanol			Dichloromethane			Chloroform			n-Hexane		
Organisms	**CIP (10 µg) (mm)**	**AMI**	**PAI**	**TAI (mL/g)**	**AMI**	**PAI**	**TAI (mL/g)**	**AMI**	**PAI**	**TAI (mL/g)**	**AMI**	**PAI**	**TAI (mL/g)**	**AMI**	**PAI**	**TAI (mL/g)**
S. aureus	28	0.54	54	6.98	0.51	51	5.10	0.44	44	3.47	0.42	42	2.33	0.33	33	1.31
S. saprophyticus	25	0.72	72	8.14	0.68	68	5.95	0.61	61	3.95	0.55	55	2.52	0.47	47	1.60
S. epidermidis	21	1.00	100	8.72	0.94	94	6.38	0.86	86	4.33	0.83	83	2.89	0.63	63	1.89
S. aureus (MRSA) ATCC 33591	16	0.93	93	6.43	0.86	86	4.70	0.82	82	3.37	0.73	73	2.27	0.51	51	1.23
B. subtilis	26	1.11	111	42.75	1.06	106	31.25	0.98	98	8.94	0.98	98	5.62	0.79	79	3.00
B. cereus	22	1.23	123	37.17	1.16	116	27.17	1.17	117	13.12	1.07	107	8.30	0.93	93	3.00
M. luteus	20	1.26	126	27.58	1.16	116	20.16	1.16	116	8.08	1.07	107	5.11	0.90	90	2.38
C. xerosis	19	1.53	153	48.86	1.48	148	35.71	1.44	144	10.41	1.39	139	6.76	1.15	115	3.04
VRE E. faecalis (ATCC 51299)	16	0.94	94	7.57	0.87	87	5.53	0.73	73	3.29	0.67	67	2.24	0.52	52	1.23
E. faecalis	20	0.82	82	9.50	0.71	71	6.94	0.61	61	3.33	0.62	62	2.25	0.48	48	1.48
Vibrio sp.	16	0.89	89	7.10	0.79	79	5.19	0.68	68	2.85	0.67	67	1.90	0.63	63	1.62
E. coli	22	1.10	110	81.43	1.10	110	59.52	1.07	107	7.83	1.02	102	5.03	0.91	91	2.80
K. pneumoniae	20	1.00	100	20.36	0.90	90	14.88	0.85	85	6.39	0.79	79	4.20	0.80	80	2.36
Shigella sp.	22	1.19	119	28.98	1.10	110	21.19	1.05	105	7.65	1.00	100	5.53	0.85	85	2.68
P. aeruginosa	19	1.02	102	16.29	0.94	94	11.90	0.89	89	6.64	0.84	84	4.24	0.75	75	2.77
P. mirabilis	20	1.07	107	14.87	1.01	101	10.87	0.91	91	5.94	0.87	87	3.84	0.86	86	2.10
Enterobacter sp.	20	1.00	100	12.13	0.94	94	8.87	0.84	84	5.37	0.79	79	3.63	0.83	83	2.32
S. typhi	17	1.08	108	10.49	0.95	95	7.67	0.84	84	3.81	0.78	78	2.49	0.69	69	1.55
S. paratyphi A	16	0.97	97	9.77	0.86	86	7.14	0.74	74	3.41	0.74	74	2.32	0.60	60	1.36
S. paratyphi B	20	0.79	79	8.55	0.70	70	6.25	0.63	63	3.09	0.59	59	2.04	0.41	41	1.30

Table S5. Antifungal activity indicating zone of inhibition (ZOI), minimal inhibitory and fungicidal concentrations (MICs and MFCs) of different solvents extracts of whole body of *C. perezi*

Organisms	ZOI (mm)±SD					MIC & MBC (µg /mL)±SD				
	Water	**Methanol**	**Dichloromethane**	**Chloroform**	**n-Hexane**	**Water**	**Methanol**	**Dichloromethane**	**Chloroform**	**n-Hexane**
T. mentagrophytes	25.0±0.57^{b}	26.1±0.42^{a}	24.8±0.76^{c}	22.9±0.42^{d}	19.8±0.57^{e}	11.80±0.57^{d}	10.90±0.74^{e}	13.30±0.84^{c}	14.70±0.67^{b}	15.80±0.57^{a}
M. gypseum	23.0±0.76^{b}	24.2±0.76^{a}	22.4±0.42^{c}	20.8±0.57^{d}	16.2±0.76^{e}	14.20±0.76^{d}	13.00±0.79^{e}	14.80±0.57^{c}	16.00±0.79^{b}	21.20±0.76^{a}
A. flavus	26.0±0.96^{b}	27.3±0.84^{a}	25.7±0.57^{c}	24.0±0.61^{d}	18.4±0.96^{e}	08.00±1.00^{d}	07.60±0.74^{e}	08.90±0.74^{c}	10.00±0.79^{b}	16.50±1.00^{a}
A. niger	27.0±0.65^{b}	28.2±0.57^{a}	26.8±0.57^{c}	26.2±0.76^{d}	15.4±0.65^{e}	06.60±0.42^{d}	05.90±0.74^{e}	07.90±0.74^{c}	08.80±0.57^{b}	20.60±0.42^{a}
A. terreus	24.5±0.57^{b}	25.0±0.79^{a}	22.7±0.67^{c}	21.5±0.50^{d}	13.2±0.57^{e}	13.10±0.74^{d}	11.70±0.57^{e}	14.10±0.65^{c}	15.00±0.61^{b}	23.10±0.74^{a}
A. terricola	19.7±0.79^{b}	20.9±0.74^{a}	19.0±0.61^{c}	18.4±0.42^{d}	12.0±0.79^{e}	16.60±1.14^{d}	15.00±0.79^{e}	17.20±0.57^{c}	19.40±1.14^{b}	24.60±1.14^{a}
Chrysosporium sp.	23.0±0.79^{b}	24.2±0.57^{a}	22.5±0.65^{c}	21.7±1.04^{d}	15.0 ± 0.79^{e}	08.00±0.65^{d}	07.00±0.79^{e}	09.20±0.57^{c}	11.00 ± 0.79^{b}	15.60 ± 0.65^{a}
F. oxysporum	19.0±0.79^{b}	20.3±0.84^{a}	18.5±0.76^{c}	17.7±0.57^{d}	14.0±0.79^{e}	16.80±0.57^{d}	15.70±0.84^{e}	19.00±0.79^{c}	21.40±0.96^{b}	23.80 ± 0.57^{a}
P. variotii	17.5±0.82^{b}	18.3±0.57^{a}	16.9±0.65^{c}	15.5±0.61^{d}	11.1±0.82^{e}	21.40±0.96^{d}	20.00±0.79^{e}	24.00±0.79^{c}	27.20±0.76^{b}	27.40±0.96^{a}
Penicillium sp.	17.0±0.79^{b}	18.7±1.20^{a}	16.4±0.96^{c}	14.6±0.65^{d}	12.0±0.79^{e}	26.60±0.65^{d}	25.80±0.76^{e}	28.00±0.79^{c}	29.60±0.65^{b}	31.60±0.65^{a}
C. albicans	27.5±0.89^{b}	28.6±0.65^{a}	26.9±0.65^{c}	25.5±0.87^{d}	19.9±0.89^{e}	03.10±0.89^{d}	02.60±0.42^{e}	04.30±0.57^{c}	05.90±0.74^{b}	11.10±0.89^{a}
S. cerevisiae	28.6±0.65^{b}	29.7±0.57^{a}	28.0±0.65^{c}	27.2±0.76^{d}	21.9±0.65^{e}	02.80±0.76^{d}	02.40±0.42^{e}	03.80±0.57^{c}	05.20±1.04^{b}	09.80±0.76^{a}

Data (±SD, n=9) followed by different letters (superscripts) indicate significant differences among variables ($a = p < .001$; $b = p < .01$; c, d,$e = p < .05$)

Table S6. Antimicrobial index (AMI), percentage activity index (PAI) and total activity index (TAI) of different solvents extracts of whole body of *C. perezi*

Organisms	MIZ (10 µg) (mm)	Water			Methanol			Dichloromethane			Chloroform			n-Hexane		
		AMI	PAI	TAI (mL/g)	AMI	PAI	TAI (mL/g)	AMI	PAI	TAI (mL/g)	AMI	PAI	TAI (mL/g)	AMI	PAI	TAI (mL/g)
T. mentagrophytes	23	1.08	108	10.82	1.13	113	11.47	1.08	108	7.59	1.00	100	4.97	0.86	86	3.29
M. gypseum	22	1.05	105	8.07	1.10	110	9.62	1.02	102	6.82	0.95	95	4.56	0.74	74	2.83
A. flavus	27	0.96	96	10.36	1.01	101	16.45	0.95	95	11.35	0.89	89	7.30	0.68	68	4.64
A. niger	29	0.93	93	8.30	0.97	97	21.19	0.92	92	12.78	0.90	90	8.30	0.53	53	4.95
A. terreus	21	1.17	117	7.40	1.19	119	10.68	1.08	108	7.16	1.02	102	4.87	0.63	63	3.04
A. terricola	21	0.94	94	6.95	1.00	100	8.33	0.90	90	5.87	0.88	88	3.76	0.57	57	2.46
Chrysosporium sp.	25	0.92	92	10.96	0.97	97	17.86	0.90	90	10.98	0.87	87	6.64	0.60	60	4.05
F. oxysporum	26	0.73	73	7.18	0.78	78	7.96	0.71	71	5.32	0.68	68	3.41	0.54	54	2.14
P. variotii	24	0.73	73	6.24	0.76	76	6.25	0.70	70	4.21	0.65	65	2.68	0.46	46	1.75
Penicillium sp.	24	0.71	71	5.41	0.78	78	4.84	0.68	68	3.74	0.61	61	2.47	0.50	50	1.55
C. albicans	28	0.98	98	15.41	1.02	102	78.13	0.96	96	23.49	0.91	91	12.37	0.71	71	6.46
S. cerevisiae	27	1.06	106	17.45	1.10	110	89.29	1.03	103	26.58	1.01	101	14.04	0.81	81	6.71