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In vitro antioxidant and enzyme inhibitory studies, computational analysis and chemodiversity of an emergency food plant *Caralluma edulis* (Edgew.) Benth. ex Hook.f: A multifunctional approach to provide new ingredients for nutraceuticals and functional foods

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ABSTRACT

Caralluma edulis is a leafless succulent herb native to India and Pakistan, where it is used as emergency food and as potential herbal medicine. It was investigated for its bioactive chemicals and further medicinal properties. The objective of the current study was to accomplish the chemical and biological characterization of *C. edulis*. Chemical profiling was done through estimation of total bioactive contents and UHPLMS analysis. Biological screening was achieved through six different antioxidant and enzyme inhibition assays. Estimation of its phenolic and flavonoid contents revealed that ethyl acetate fraction (Ce-E) contains 14.15 mg GAE/g extract and 26.13 mg RE/g extract of phenolics and flavonoids, respectively, followed by the methanolic (Ce-M) and the water (Ce-W) soluble fractions. In free radical inhibitory assays, Ce-E fraction also exhibited highest activity (DPPH: 19.72 mg TE/g extract, ABTS: 48.66 mg TE/g extract) followed by Ce-M, whereas, in CUPRAC and FRAP assays, all the extracts, except water fraction, exhibited nearly equal potential in the range of 53.85–57.96 and 25.42–32.12 mg TE/g extract, respectively. In metal chelating antioxidant assay, only Ce-M and Ce-W fractions displayed considerable activities (25.88 and 23.55 mg EDTAE/g extract respectively), whereas, Ce-H exhibited the highest activity in phosphomolybdenum assay (1.03 mmolTE/g extract), against BChE (6.43 mg GALAE/g extract), α-glucosidase and α-amylase (6.98 and 0.47 mmol ACAE/g, respectively) and tyrosinase (64.46 mg KAE/g extract) enzymes. Ce-M and Ce-E also exhibited significant activity against tyrosinase (59.85 and 58.40 mg KAE/g extract, respectively). Crude methanolic extract (Ce-M) was analyzed to unveil its secondary metabolic picture; the UHPLCMS analysis discloses 105 compounds as phenolic acids, flavonoids, steroids and their glycosides, which makes the *C. edulis* extract a strong candidate for nutraceutical and functional food ingredient. Some of the metabolites were docked against all the tested enzymes to predict mode of actions and to substantiate the pharmaceutical nature of *C. edulis*.

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1. Introduction

Increasing demand of food and continuous increase in food prices have developed a serious global food security concern. As an estimate, about 100 million people are thought to be suffered by absolute poverty since 2007. Most of them reside in developing nations where access to food is difficult due to low income (less than $1 per day). Food security is the ability to obtain enough food to meet one's dietary demands and food preferences. However, while price increases have raised attention to food security, fundamental issues still need to be addressed (Flood, 2010). Therefore, investigation on emergency food plants is important aspect and how they might be used as reliable sources of food and medication.

Caralluma plants of the family Asclepiadaceae are perennial, small and usually leafless mostly cactus type herbs widely distributed in Asia, Africa, Arabian Peninsula, Canary Islands and Southeast Europe; some of these plants are succulent and are edible (Marwah et al., 2007). In several folkloric medicinal systems Caralluma plants are being used for the treatment of diabetic and rheumatic patients. In India and Pakistan, Caralluma species have been used as emergency foods (Sireesha, 2018) therefore, Caralluma species are now gaining much attention for being food crop and because of its immunostimulating activities, attributed to the presence of flavonoids, saponins, pregnane glycosides (Sireesha, 2018), megastigmane glycosides and various esters, which authenticate its medicinal potential (Adnan et al., 2014). Further, various pharmacological studies disclosed antioxidant, anticancer, antidiabetic, anti-inflammatory, antimicrobial, anti-eczemic, antimalarial and antifungal properties of *Caralluma* extracts (Waheed et al., 2011). Al-Faifi et al. have reported the alcoholic extracts of *C. acutangula* as significant inhibitor of hepatocellular carcinoma cell and MCF7 cancer cell lines (Al-Faifi et al., 2016). *C. fimbriata* aqueous alcohol extract was investigated for its renoprotective effect on Wistar rats (Gujjala et al., 2016), while aqueous methanolic extract of *C. tuberculata* has been disclosed to afford antihypertensive activity (Ahmad et al., 2015). Different extracts of *C. quadrangular* offered their properties as antibiotics against *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus luteus* and *Candida albicans* (Farouk et al., 2016).

C. edulis (Edgew.) Benth. ex Hook.f. is an important member of the genus *Caralluma* (Ahmad et al., 2009). It is an erect succulent herb growing up to 15–45 cm in height and is considered as native to Indian and Pakistan where its succulent stems are eaten raw (Ansari et al., 2022; Hooker, 1885). Some documents describe *C. edulis*, in Indian, as a rare and endemic plant growing in Thar Desert, where it is popular for its medicinal and edible properties and high anti-oxidant potential (Parihar, 2016).

In folk medicine system, *C. edulis* is used against parasitic infections, Alzheimer disease, rheumatism, hypertension, gastric disorders, leprosy, diabetes (Adnan et al., 2014; Wadood et al., 1989) and is used as anti-oxidant (Ansari et al., 2005). Other studies disclosed *C. edulis* as anti-inflammatory plant and thus can be used against rheumatism (Minhas et al., 2018). In addition, *C. edulis* possesses anti-nociceptive property, thus can be used as analgesic drug (Firdoos et al., 2017). In another study, C. edulis has been reported to possess antihyperlipidemic effect, thus can have therapeutic potentials for the management of lipid disorders (Ashfaq et al., 2017). Literature reports revealed that *C. edulis* contains saponins, alkaloids, tannins, phenol, glycosides, terpenoids and flavonoids (Ashfaq et al., 2017). In addition, previously eighteen fatty acids, four hydrocarbons and sterol have also been isolated from the ethyl acetate fraction of the vegetative part of *C. edulis* (Rizwani et al., 1993).

Owing to the medicinal importance of *Caralluma* plants, the present work was planned to determine complete phytochemical composition, antioxidant potential and enzyme inhibition properties of the extracts of *C. edulis*. Methanolic extract (Ce-M) was divided into hexane (Ce-H), ethyl acetate (Ce-E) and water (Ce-W) soluble fractions, which were subjected to the measurement of their phenolic and flavonoid contents, whereas, to unveil the complete secondary metabolic picture of *C. edulis*, the total methanolic extract (Ce-M) was analyzed by HPLC-MS. Various bioassays (DPPH, CUPRAC, ABTS, FRAP, Phosphomolybdenum and metal chelation assays) were performed to measure antioxidant potential of the extracts, whereas, all the fractions were also evaluated against key enzymes involved in common ailments like diabetes (α-glucosidase and α-amylase) neuro-degenerative disorder (AChE and BChE) and skin problems (tyrosinase). Statistical and docking studies were also performed to highlight possible interaction between the bioactive content and tested biological assays.

2. Materials and methods

2.1. Plant material and extraction

Whole plant of *C. edulis* was collected from Cholistan Desert in May 2019, which was identified by Dr. Farrukh Nisar, plant taxonomist, Department of Biochemistry, Cholistan University of Veterinary and Animal Sciences, Bahawalpur. A Voucher specimen No. CE-2-5-19 has been deposited in the herbarium.

The whole plant material (5.0 Kg) was dried under shade for 15 days, chopped into small pieces and was macerated (twice, for 07 days each) with distilled methanol (7.0 L). The solvent was evaporated under reduced pressure to get a dark green gummy extract (Ce-M, 105 g). 100 g of the Ce-M was suspended in distilled water (500 mL) and extracted with *n*-hexane and ethyl acetate to get 25 g of hexane (Ce-H) and 22 g of ethyl acetate (Ce-E) soluble fractions, respectively, whereas aqueous layer (Ce-W) was dried up to 45 g of the residue.

2.2. Determination of total phenolic and flavonoid contents

The total phenolic contents (TPC) were measured by Folin-Ciocalteu protocol, while total flavonoid contents (TFC) were estimated in aluminum chloride assays (Slinkard and Singleton, 1977; Tousif et al., 2022) In these analyses, flavonoid and phenolic contents are expressed as rutin (mg RE/g extract) and gallic acid (mg GAEs/g extract) equivalents respectively.

2.3. Antioxidant assays

Already established protocols (Grochowski et al., 2017) were followed to estimate antioxidant activity of the extracts. FRAP, ABTS, DPPH, CUPRAC and total antioxidant capacity were expressed as trolox equivalent while ethylene diamine tetra acetic acid (EDTA) was used as reference for metal chelating assay.

2.4. Enzyme inhibition assays

AChE, BChE, tyrosinase, α-amylase and α-glucosidase enzyme inhibitory assays were also performed according to reported methods in literature (Mollica et al., 2017; Zubair et al., 2022) AChE and BChE inhibitory activity was measured as equivalent of galantamine (mg GALAE/g extract), α-amylase and α-glucosidase inhibitory potential as equivalent of acarbose (mmol ACAE/g extract), while for tyrosinase activity kojic acid (mmol KAE/g extract) was used as reference drug.

2.5. Docking analysis

Some of the major class-identified molecules in the extracts were computationally screened through molecular docking, to measure binding affinity of these compounds to the targeted enzymes. The activity was measured in terms of binding affinity (Kcal/mol) using the Auto dock 4.2 software (Morris et al., 2009), and the results were then contrasted in terms of binding affinity score for the best-docked conformation. The structures of the selected compounds were sketched using Chem Draw Ultra 16.0 and Chem3D Ultra 16.0, and then

Table 1
Total phenolic (TPC) and total flavonoid (TFC) contents of *C. edulis*.

Extract/ Fraction	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg RE/g extract)
Ce-M	12.42 ± 0.03^{b}	15.77 ± 0.55^{b}
Ce-H	13.46 ± 0.81^{ab}	11.86 ± 0.48^{c}
Ce-E	14.15 ± 0.13^{a}	26.13 ± 0.40^{a}
Ce-W	10.19 ± 0.06^{c}	0.50 ± 0.04^{d}

Ce-M = Methanolic extract of *C. edulis*, Ce-H = *n*-hexane extract of *C. edulis*, Ce-E = Ethyl acetate extract of *C. edulis,* Ce-W= Water extract of *C. edulis*.
*Values expressed are means ± S.D. of three parallel measurements. GAE: Gallic acid equivalent; RE: Rutin equivalent. Different letters indicate differences in the extracts ($p < 0.05$).

the 3D pdb structures were generated. At the ADT interface, all the planned structures were translated to readable format i.e. pdbqt after being optimized by energy minimization using the MMFF94 approach. The protein structure for AChE, BChE, α-glucosidase, α-amylase and tyrosinase enzymes were retrieved from the Protein Data Bank (Sussman et al., 1998). Using Auto Dock 4.2, a program that employs an empirical grading system based on the free energy of binding, molecular docking was carried out. We selected the Lamarckian Genetic Method (LGA), a stochastic search algorithm from the Auto Dock package that combines local and global search. Using the graphical user interface tool Auto Dock tools, intermediary tasks were carried out, such as creating grid boxes and creating pdbqt files for protein and ligand preparation (ADT). The protein was given Kollman charges, polar hydrogens and merging non-polar hydrogens via ADT. The prepared file was saved by Auto Dock in pdbqt format. The grid map was created using a grid box using Autogrid. The grid size was set at 70 70 70 xyz points with grid spacing of 0.375. Utilizing protein and ligand information as well as grid box parameters from the configuration file, docking was performed using Lamarckian Genetic Algorithm. The maximum 250 runs were performed for docking while all other parameters were used as default. The enzymatic proteins were treated as stiff throughout the docking process while ligand was allowed to move within active site. The outcomes with positional root-mean-square deviation (RMSD) less than 1.0 were grouped together and represented by the outcome with the best binding free energy. For additional study, the posture with the lowest binding energy or affinity was extracted and matched with the reference structure. The analysis of the docking results was performed via Discovery Studio Visualizer (Biovia, 2017, p. 936).

2.6. Statistical analysis

ANOVA (Tukey's test) was used to determine if there were any differences in extract levels between extracts. The Pearson correlation test was used to examine the relationship between total bioactive components and biological activities. The correlation analysis was performed by using GraphPad 9.0. Additionally, Principal Component Analysis (PCA) was used to determine whether the tested extracts were similar or different. SIMCA was used to carry out the PCA analysis (version 14.0).

3. Results and discussion

3.1. Total phenolic and flavonoid contents

It is now an established fact that phenolics and flavonoids are the phytochemicals that exhibit potent antioxidants, anticancer, antibacterial, cardioprotective, anti-inflammation, immune system promoting, skin protection activities, and thus the plats rich in these metabolites are attractive sources for pharmaceutical and functional food applications (Chen et al., 2015; Meng et al., 2018; Nourozi et al., 2019). Nutraceutical and functional food formulations comprising phenolic and flavonoid rich herbal extracts promote human health, prevent and cure various diseases (Chandra et al., 2014; de Araújo et al., 2021; Tungmunnithum et al., 2018). Keeping in view these facts, and use of *C. edulis* as emergency food, we investigated its methanolic extract and different fractions for their phenolic and flavonoid contents. The investigation revealed the highest phenolic contents in ethyl acetate fraction (Ce-E, 14.15 mg GAE/g extract) followed by Ce-H (13.46 mg GAE/g extract), Ce-M (12.42 mg GAE/g extract) and Ce-W (10.19 mg GAE/g extract) (Table 1). Similarly, the highest flavonoid contents were also found in Ce-E with value 26.13 mg RE/g extract, followed by Ce-M (15.77 ± 0.55 mg RE/g extract and Ce-H (11.86 ± 0.48 mg RE/g extract). Water fraction (Ce-W) was poor carrier of phenolics and flavonoids (Table 1). The published data on chemical profiling of several other species of the *Caralluma* genus was comparable to our results (Bourhia et al., 2020; Chandran et al., 2014), however, usually methanolic or alcoholic extracts are reported rich in phenolics and flavonoids followed by ethyl acetate and aqueous extracts. In the present study, Ce-E was found rich in phenolics and flavonoids, followed by Ce-M; the slight difference could be attributed to extraction techniques, because in most published work, the plant material was extracted separately with different solvents, but in the present work the plant material was extracted with methanol, which in turn was divided into hexane, ethyl acetate and water soluble parts.

Phenolic compounds have the power to act as antioxidant and to eliminate reactive oxygen species (ROS) in living cells. In addition to several biological properties, phenolics and flavonoids protect against oxidative stress by lowering oxidation of low density lipoproteins (Huyut et al., 2017; Snezhkina et al., 2019). Since all the above tested fractions, especially Ce-E are rich in phenolic compounds, it makes *C. edulis* a strong candidate to be added in nutraceutical and functional food formulations.

3.2. Chemical profiling: identification of secondary metabolites

UHPLC-MS analysis of the crude methanolic extract (Ce-M) resulted in identification of 105 compounds (Table 2) mostly of steroidal saponin, megastigmane glycosides, triterpenes, phenolic and flavonoids classes as reported in literature for other members of *Caralluma* (Adnan et al., 2014). Previously, *C. edulis* has never been chemically investigated or isolation of phytochemicals, in the context of phytochemical investigation, this work authenticates its use as emergency food and discloses its candidature as nutraceutical or functional food ingredient. Furthermore, identification of high amount of steroid make this plant effective in the treatment of rheumatism, diabetes, and leprosy, as well as acting as antiseptics and disinfectants (Abdel-Sattar et al., 2007). In addition to triterpenoids have recently emerged as a significant group of secondary metabolites with a wide range of therapeutic effects, including anti-inflammatory, antioxidant, antibacterial, antiviral, hepatoprotective, gastroprotective, cardioprotective, hypolipidemic, antiatherosclerotic, immunoregulatory and anticancer (Rascon-Valenzuela et al., 2017). Further the presence of phenolic in higher concentration, as well as flavonoids and make this plant more important (Khasawneh et al., 2014).

3.3. Antioxidant assays

Since, *C. edulis* extracts constitute significant amount of phenolics and flavonoids, it was reasonable to evaluate these extracts for their complete antioxidant potential. To get picture of true antioxidant property of *C. edulis*, its extract and fractions were tested in different antioxidant capacities including DPPH, ABTS, FRAP, CUPRAC, phosphomolybdenum and metal chelating assays. The results are presented in Table 3, which discloses that Ce-E showed the highest radical scavenging activity (DPPH: 19.72 mgTE/g and ABTS: 48.66 mgTE/g respectively), followed by Ce-M (Table 3). These results are in accord with the highest total phenolic and flavonoid content present in Ce-E (Table 1). Previous studies by Khasawneh on *C. arabica* also reported highest antioxidant activity of the ethyl acetate extracts, which was

Tables 2
Identification of secondary metabolites through UHPLCMS analysis.

Sr. No.	Analyte Peak	Retention Time	Area/ Height	Identified compounds	class	Formula	M. mass
1	429.2146	1.51	6.28	Taraxacolide 1-O- β-D-glucopyranoside	Sesquiterpene	$C_{21}H_{32}O_9$	428.2046
2	349.0663 (M + K+)	1.52	6.47	(E)-1-O-Cinnamoyl-β-D-glucose	Glycoside	$C_{15}H_{18}O_7$	310.1053
3	365.0977	1.84	3.50	Robustone	Isoflavone	$C_{21}H_{16}O_6$	364.0947
4	333.0930 (M + Na+)	2.76	9.49	Dihydrotricetin 7,3′-dimethyl ether	Flavonone	$C_{17}H_{16}O_7$	332.0896
5	307.0781	3.18	14.05	Epigallocatechin	Flavonoid	$C_{15}H_{14}O_7$	306.0740
6	291.0641 (M + K+)	3.35	11.16	Methyl 3,4,5-trimethoxycinnamate	Coumaric acid	$C_{13}H_{16}O_5$	252.0998
7	305.0988	3.78	11.66	4′-O-Methylcatechin	Polyphenol	$C_{16}H_{16}O_6$	304.0947
8	303.0833	3.83	10.39	3′,5,7-Trihydroxy-3′-methoxyflavanone, Homoeriodictyol	Flavonoid	$C_{16}H_{14}O_6$	302.0790
9	346.1477 (M + NH4+)	3.81	10.48	3-(4-Hydroxyphenyl)-3-oxopropyl- β-D-glucopyranoside	Glycoside	$C_{15}H_{20}O_8$	328.1158
10	422.1467	3.91	8.13	5-O-p-Coumaroylnigrumin	Phenolics (tannins)	$C_{20}H_{23}NO_9$	421.1373
11	399.1795	3.90	5.55	3β,4β,5-Trimethoxy-4′-hydroxy- (6:7)-2,2-dimethylpyranoflavan	Flavonoid	$C_{23}H_{26}O_6$	398.1729
12	291.0837	3.98	7.94	Nubigenol	Chalcones	$C_{15}H_{14}O_6$	290.0790
13	303.0833	4.28	10.04	5,7,8-Trihydroxy-4′-methoxyflavanone	Flavonones	$C_{16}H_{14}O_6$	302.0790
14	288.1594 (M + NH4+)	4.40	5.87	trismethoxy Resveratrol	Polyphenol	$C_{17}H_{18}O_3$	270.1256
15	270.1340 (M + NH4+)	4.47	8.05	Methyl 3,4,5-trimethoxycinnamate	Methoxy benzenes	$C_{13}H_{16}O_5$	252.0998
16	369.1127	4.55	6.82	3-O-Feruloylquinic acid	Aromatic carboxylic acid	$C_{17}H_{20}O_9$	368.1107
17	371.0921	4.50	6.93	5-Hydroxy-6-methoxycoumarin 7-glucoside	Coumarin	$C_{16}H_{18}O_{10}$	370.0900
18	363.1388 (M + Na+)	4.54	5.33	2-Prenylhydroquinone-1-glucoside	Hydroquinone glycoside	$C_{17}H_{24}O_7$	340.1522
19	417.1908	4.77	5.99	Leonubiastrin	Diterpenoid	$C_{23}H_{28}O_7$	416.1835
20	337.1399 (M + Na+)	4.85	5.96	Anisocoumarin H	Coumarin	$C_{19}H_{22}O_4$	314.1518
21	387.1998	4.91	3.97	Corchoionol C 9-glucoside	Glycoside	$C_{19}H_{30}O_8$	386.1941
22	403.2301 (M + CH3OH + H+)	4.93	4.60	(3S,7E,9S)-9-Hydroxy-4,7-megastigmadien-3-one 9-glucoside	Glycoside	$C_{19}H_{30}O_7$	370.1992
23	391.1331 (M + Na+)	4.85	6.36	4-Hydroxy-3-prenylbenzoic acid glucoside	Glycoside	$C_{18}H_{24}O_8$	368.1471
24	281.1386	4.84	5.46	13-Hydroxyabscisic acid	Sesquiterpenoid	$C_{15}H_{20}O_5$	280.1311
25	402.2332 (M + NH4+)	4.93	4.71	16-Methoxy-2,3-dihydro-3-hydroxytabersonine	Alkaloid	$C_{22}H_{28}N_2O_4$	384.2049
26	461.2160	5.09	8.41	17,21-Dihydroxypregn-4-ene-3,11,20-trione 21-(hydrogensuccinate)	Corticosteroid	$C_{25}H_{32}O_8$	460.2097
27	323.1638	5.27	5.84	Derricin	Chalcones	$C_{21}H_{22}O_3$	322.1569
28	342.1598	5.29	8.54	5,8,13,13α-Tetrahydrocolumbamine	Alkaloid	$C_{20}H_{23}NO_4$	341.1627
29	447.2373	5.25	5.18	Semimyrtucommulone	Phenol	$C_{25}H_{34}O_7$	446.2305
30	404.2241 (M + NH4+)	5.34	5.23	Citroside A	Terpene glycoside	$C_{19}H_{30}O_8$	386.1941
31	577.2623	5.36	6.02	Ptilosaponoside B	Steroid	$C_{27}H_{44}O_{11}S$	576.2604
32	285.0728	5.39	4.37	Thevetiaflavone	Flavones	$C_{16}H_{12}O_5$	284.0685
33	335.1098	5.38	8.32	Mesquitol-4β-ol 3,8-dimethyl ether	Flavonoid	$C_{17}H_{18}O_7$	334.1053
34	301.1030	5.60	15.15	2′,4′-Dihydroxy-3′,6′-dimethoxychalcone	Flavonoid	$C_{17}H_{16}O_5$	300.0998
35	344.2043 (M + NH4+)	5.45	5.24	5-Acetoxy-3-(hydroxymethyl)-6-isopropyl-2-oxo-3-cyclohexen-1-yl 3-methylbutanoate	Phyto	$C_{17}H_{26}O_6$	326.1649
36	561.1655	5.49	1.38	Cassiaoccidentalin A	Flavanoidal glycoside	$C_{27}H_{28}O_{13}$	560.1530
37	492.2639 (M + NH4+)	5.53	4.75	Flemiphyllin	Isoflavanoid	$C_{30}H_{34}O_5$	474.2406
38	448.2675 (M + NH4+)	5.61	5.60	21-Hydroxypregn-4-ene-3,20-dione hydrogen succinate	Steroid	$C_{25}H_{34}O_6$	430.2355
39	367.1725	5.56	4.27	4-Acetyl-2-prenylphenol glucoside	Glycoside	$C_{19}H_{26}O_7$	
40	421.2221	5.51	5.92	Armillane	Diterpenoid	$C_{23}H_{32}O_7$	420.2148
41	418.2217 (M + CH3OH + H+)	5.88	9.11	Thalicpureine	Alkaloid	$C_{22}H_{27}NO_5$	385.1889
42	317.0986	5.86	6.77	Dihydroskullcap flavone I	Flavonoid	$C_{17}H_{16}O_6$	316.0947
43	419.2424	5.89	6.89	21-Acetoxy-11β,17-dihydroxy-6α-methylpregn-4-ene-3,20-dione	Steroid	$C_{24}H_{34}O_6$	418.2355
44	435.2807	5.97	1.67	6′-Hydroxysimvastatin	Hydroxyl acid	$C_{25}H_{38}O_6$	434.2668
45	463.2679 (M + CH3OH + H+)	6.11	6.53	21-Hydroxypregn-4-ene-3,20-dione hydrogen succinate	Steroid	$C_{25}H_{34}O_6$	430.2355
46	303.1194	6.03	9.21	2′,3′-Dihydroxy-4′,6′-dimethoxydihydrochalcone	Chalcones	$C_{17}H_{18}O_5$	302.1154
47	361.1231	6.11	8.09	5,7,3′-Trihydroxy-4′,5′-dimethoxy-6,8-di-C-methylflavanone	Flavonoid	$C_{19}H_{20}O_7$	360.1209
48	465.2049	6.17	8.60	16-α,17-β-estriol 17-β-D-glucuronide	Steroid saponin	$C_{24}H_{32}O_9$	464.2046
49	479.2811	6.20	6.30	3-Geranyl-4,2′,4′,6′-tetrahydroxy-5-prenyldihydrochalcone	chalcone	$C_{30}H_{38}O_5$	478.2719
50	506.3073 (M + NH4+)	6.32	6.69	Perulactone B	Steroid	$C_{28}H_{40}O_7$	488.2774
51	491.2194	6.32	4.94	Piperaduncin A	Chalcones	$C_{29}H_{30}O_7$	490.1992
52	333.1829	6.44	5.00	(1R,3S,4S)-1,8-Epoxy-p-menthan-3-ol glucoside	Glycoside	$C_{16}H_{28}O_7$	332.1835
53	525.2603 (M + Na+)	6.37	5.64	Withaperuvin G	Steroid	$C_{28}H_{38}O_8$	502.2567
54	325.1633	6.36	9.22	Blumealactone C	Terpenoid	$C_{17}H_{24}O_6$	324.1573

(*continued on next page*)

Tables 2 (*continued*)

Sr. No.	Analyte Peak	Retention Time	Area/ Height	Identified compounds	class	Formula	M. mass
55	521.3258	6.37	5.47	cyasterone	Steroid	$C_{29}H_{44}O_8$	520.3036
56	317.1930	6.44	3.45	Neryl glucoside	Terpenoid	$C_{16}H_{28}O_6$	316.1886
57	331.1134	6.52	12.84	7-Hydroxy-5,8,2′-trimethoxyflavanone	Flavonoid	$C_{18}H_{18}O_6$	330.1103
58	369.2056	6.61	9.70	Quercetol B	Flavonoid	$C_{23}H_{28}O_4$	368.1988
59	458.2536 (M + NH4+)	6.87	8.18	Deacetylgedunin	Terpenoid	$C_{26}H_{32}O_6$	440.2199
60	389.1537 (M + Na+)	6.74	8.57	4-Acetyl-2-prenylphenol glucoside	Glycoside	$C_{19}H_{26}O_7$	366.1679
61	409.1806 (M + CH3OH + H+)	6.80	9.55	(3S)-7-hydroxy-2′,3′,4′,5′,8-pentamethoxyisoflavan	Flavonoid	$C_{20}H_{24}O_7$	376.1522
62	413.2307	6.73	15.58	3β,21-Dihydroxy-pregna-5,7,9(11)-trien-20-one diacetate	Steroid	$C_{25}H_{32}O_5$	412.2250
63	682.4053 (M + NH4+)	6.91	5.81	Medicagenic acid 3-O-β-D-glucoside	Triterpene saponins	$C_{36}H_{56}O_{11}$	664.3823
64	446.2880 (M + NH4+)	6.95	6.70	2-Angeloyl-9-(3-methyl-2E-pentenoyl)-2b,9a-dihydroxy-4Z,10(14)-oplopadien-3-one	Sesquiterpenoid	$C_{26}H_{36}O_5$	428.2563
65	697.4108	6.93	4.71	Momordicoside E	Steroid saponin	$C_{37}H_{60}O_{12}$	696.4085
66	384.1988 (M + NH4+)	7.00	11.90	Orizabin	Sesquiterpenoid	$C_{19}H_{26}O_7$	366.1679
67	389.1539 (M + Na+)	6.98	8.90	Secoeremopetasitolide A	Terpene lactone	$C_{19}H_{26}O_7$	366.1679
68	457.2633 (M + NH4+)	6.95	13.43	Militarinone B	Monoterpenoid	$C_{26}H_{33}NO_5$	439.2359
69	725.4213	7.06	4.86	Alliospiroside C	Steroid saponin	$C_{38}H_{60}O_{13}$	724.4034
70	740.4504	7.03	4.64	Lycoperoside D	Alkaloid	$C_{39}H_{65}NO_{12}$	739.4507
71	412.2470 (M + NH4+)	7.04	4.16	Gancaonin J	Chalcones	$C_{25}H_{30}O_4$	394.2144
72	357.2295	7.11	4.44	5,7-Megastigmadien-9-ol glucoside	Fatty acyls	$C_{19}H_{32}O_6$	356.2199
73	355.2088	7.10	6.76	Rauwolscine	Alkaloid	$C_{21}H_{26}N_2O_3$	354.1943
74	516.2737	7.15	9.81	Spirodihydrobenzofuranlactam IV	Monoterpenoid	$C_{28}H_{37}NO_8$	515.2519
75	505.3137	7.15	4.94	Desglucocoroloside	Steroid	$C_{29}H_{44}O_7$	504.3087
76	399.2513	7.27	12.29	Pregna-5,16,20-triene-3β,20-diol diacetate	Steroid	$C_{25}H_{34}O_4$	398.2457
77	399.2338	7.26	10.03	Desacetoxyvindoline	Alkaloid	$C_{23}H_{30}N_2O_4$	398.2206
78	548.3184 (M + NH4+)	7.28	5.99	Physapubescin	Steroid	$C_{30}H_{42}O_8$	530.2880
79	593.3827	7.38	3.34	Avenestergenin B2	Triterpenoid	$C_{37}H_{52}O_6$	592.3764
80	460.3099 (M + NH4+)	7.41	8.22	Muzanzagenin	Steroid	$C_{27}H_{38}O_5$	442.2719
81	506.2901 (M + NH4+)	7.59	10.41	α-Ionol O-[arabinosyl-(1->6)-glucoside]	Carbohydrate	$C_{24}H_{40}O_{10}$	488.2621
82	549.3383 (M + CH3OH + H+)	7.67	7.50	Cucurbitacin L	Triterpenoid	$C_{30}H_{44}O_7$	516.3087
83	463.0967	7.63	1.56	Scutellarein 7-glucuronide	Flavonoid	$C_{21}H_{18}O_{12}$	462.0798
84	695.3930	7.74	6.86	Capsoside A	Fatty acid	$C_{33}H_{58}O_{15}$	694.3776
85	361.1905 (M+2H2+)	7.81	9.19	Arvenin I	Triterpene	$C_{38}H_{56}O_{13}$	720.3721
86	310.1931 (M+2H2+)	7.86	5.11	2α,7β,15β,18-tetraacetoxy-cholest-5-en-3α-ol	steroid	$C_{35}H_{54}O_9$	618.3768
87	635.3723 (M + CH3OH + H+)	7.93	7.46	Physapruin B	Steroid	$C_{34}H_{50}O_9$	602.3455
88	598.3198 (M + Na+)	7.92	7.20	3,14,25-Trihydroxy-2,10,13,21,24-pentaoxo-3,9,14,20,25-pentaazatriacontan-30-oic acid	phyto	$C_{25}H_{45}N_5O_{10}$	575.3166
89	592.3595 (M + NH4+)	7.92	6.48	Cucurbitacin A	Triterpenoid	$C_{32}H_{46}O_9$	574.3142
90	531.2799	8.04	15.22	7,12-Dihydroxy-3,11,15,23-tetraoxolanost-8-en-26-oic acid	Triterpenoid	$C_{30}H_{42}O_8$	
91	623.3728	7.98	7.87	3-O-Protocatechuoylceanothic acid	Triterpenoid	$C_{37}H_{50}O_8$	622.3506
92	459.2567	8.09	9.37	[6]-Gingerdiol 4′-O-β-D-glucopyranoside	Carbohydrate	$C_{23}H_{38}O_9$	458.2516
93	722.4385	8.06	6.47	β-Solanine	Alkaloid	$C_{39}H_{63}NO_{11}$	721.4401
94	365.1914 (M + H + Na2+)	8.14	7.53	Hovenidulcioside A2	Diterpenoid	$C_{38}H_{58}O_{12}$	706.3928
95	666.3941	8.06	6.85	1-Palmitoyl-2-(5-hydroxy-8-oxo-6-octenedioyl)-sn-glycero-3-phosphatidylcholine	Lipids	$C_{32}H_{60}NO_{11}P$	665.3904
96	487.1869 (M + Na+)	8.32	5.11	Isogemichalcone B	Chalcones	$C_{29}H_{26}O_7$	486.1679
97	949.5370	8.38	4.76	Momordicoside B	Triterpenoid	$C_{47}H_{80}O_{19}$	948.5294
98	593.2054/9.27	9.37	2.38	Osmanthuside B	Carboxylic ester	$C_{29}H_{36}O_{13}$	592.2156
99	531.3789/10.73 M + H+	10.80	12.88	Acinospesigenin A	Triterpene	$C_{32}H_{50}O_6$	530.3607
100	495.3191/10.87 M + H+	10.81	14.03	makisterone B	Steroid	$C_{28}H_{46}O_7$	494.3244
101	517.3587/11.26 M + Na+	11.30	4.91	28-Homobrassinolide	Steroid	$C_{29}H_{50}O_6$	494.3607
102	451.3363/11.35	11.35	10.65	3α,7α,16α-Trihydroxy-5β-cholestan-26-oic acid	Steroid	$C_{27}H_{46}O_5$	450.3345
103	407.3113/11.37 M + H+	11.38	8.77	26,27-dinor-3α,6α,12α-trihydroxy-5β-cholestan-24-one	Lipid	$C_{25}H_{42}O_4$	406.3083
104	363.2864/11.38 M + H+	11.41	9.31	24-Nor-5β-chol-22-ene-3α,7α,12α-triol	Steroid	$C_{23}H_{38}O_3$	362.2821
105	425.3408/11.49	11.38	8.94	(22E,24R)-Stigmasta-4,22-diene-3,6-dione	Steroid	$C_{29}H_{44}O_2$	424.3341

Table 3
Antioxidant activities of the extracts of *C. edulis*.

Plant extracts	Radical scavenging assay		Metal ion reducing power assay		Total antioxidant activity	Ferrous ion chelation
	DPPH (mg TE/g extract)	ABTS (mg TE/g extract)	CUPRAC (mg TE/g extract)	FRAP (mg TE/g extract)	Phosphomolybdenum (mmol TE/g)	Metal chelating (mg EDTAE/g)
Ce-M	16.98 ± 0.32[a]	38.63 ± 0.53[b]	53.85 ± 2.68[a]	25.42 ± 0.34[c]	0.75 ± 0.07[b]	25.88 ± 0.10[a]
Ce-H	7.22 ± 0.11[c]	16.53 ± 0.63[d]	57.84 ± 0.58[a]	28.84 ± 1.48[b]	1.03 ± 0.06[a]	na**
Ce-E	19.72 ± 0.40[b]	48.66 ± 1.29[a]	57.96 ± 2.45[a]	32.12 ± 1.26[a]	0.78 ± 0.04[b]	3.17 ± 0.46[c]
Ce-W	7.09 ± 0.01[c]	31.98 ± 0.65[c]	27.91 ± 0.10[b]	17.21 ± 0.16[d]	0.12 ± 0.02[c]	23.55 ± 0.20[b]

* Values expressed are means ± S.D. of three parallel measurements. TE: Trolox equivalent; EDTAE: EDTA equivalent. Different letters indicate differences in the extracts ($p < 0.05$)** Not active.

Table 4
Enzyme inhibition activities of the extracts of *C. edulis*.

Plant Extracts	AChE (mg GALAE/g extract)	BChE (mg GALAE/g extract)	α-Glucosidase (mmol ACAE/g extract)	α-Amylase (mmol ACAE/g extract)	Tyrosinase (mg KAE/g extract)
Ce-M	1.94 ± 0.09[c]	3.12 ± 0.50[b]	na	0.35 ± 0.01[b]	59.85 ± 0.42[b]
Ce-H	2.04 ± 0.30[ab]	6.43 ± 0.95[a]	6.98 ± 1.06[a]	0.47 ± 0.01[a]	64.46 ± 0.69[a]
Ce-E	2.37 ± 0.04[a]	4.40 ± 0.35[b]	4.21 ± 0.55[b]	0.32 ± 0.01[c]	58.40 ± 0.38[b]
Ce-W	na	na	na	0.05 ± 0.00[d]	3.19 ± 0.93[c]

*Values expressed are means ± S.D. of three parallel measurements, GALAE: Galatamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active. Different letters indicate differences in the extracts ($p < 0.05$).

attributed to the high phenolic content (Khasawneh et al., 2014), thus substantiate our studies.

On the other hand, the metal ion reducing power of the plant extracts was determined by FRAP and CUPRAC assays. In these assays cupric and ferric ions are reduced to cuprous and ferrous ions in the presence of radical scavenging agents (Zengin et al., 2016). Interestingly, Ce-E and Ce-H exhibited higher potential in both the assays (CUPRAC: 57.96 ± 2.45 and 57.84 ± 0.58 mg TE/g extract, respectively and FRAP: 32.12 ± 1.26 and 28.84 ± 1.48 mg TE/g extract, respectively) with slight difference in the values, while Ce-M and Ce-W also exhibited less but significant potential with smaller differences (Table 3). These results prompted to conclude that in addition to phenolic compounds, other metabolites must also be involved in metal ion reducing activities (Santos-Sánchez et al., 2019).

Antioxidant potential of *C. edulis* extracts was further measured through the metal chelating assay, where ferrozine can quantitatively chelate Fe(II) and red colored complex is formed. The antioxidant components from plant extracts chelate with metal ion forming a complex (chelating activity) and prevent electron transfer. Thus oxidation reactions are halted and free radicals are not generated (Mocan et al., 2018). In the present study, the iron chelating activity of Ce-M and Ce-W was nearly equal (25.88 ± 0.10 and 23.55 ± 0.20 mgEDTAE/g extract), whereas, Ce-H was inactive while weak activity was observed due to Ce-E (Table 3). The total antioxidant capacity of all the fractions of *C. edulis* was analyzed through phosphomolybdenum assays, where the test substance (antioxidant) reduces Mo(IV) to Mo(V) green phosphate compounds which shows absorption maxima at 695 nm (Sarikurkcu et al., 2015). In this assay, highest activity was observed for Ce-H (1.03 ± 0.06 mmol TE/g) followed by Ce-E (0.78 ± 0.04 mmol TE/g) and Ce-M (0.75 ± 0.07 mmol TE/g) while Ce-W (0.12 mmol TE/g) exhibited lowest potential (Table 3).

Antioxidants from raw natural resources are always welcomed as compared to synthesized drugs, for natural products possess fewer side effects and mostly work in synergism. Under physiological conditions, the concentrations of ROS are usually regulated by antioxidants, which can be either generated endogenously or externally supplemented. Antioxidant supplementation has been shown to attenuate endogenous antioxidant depletion thus alleviating associated oxidative damage (Liu et al., 2018). In recent times, herbs having antioxidant potential are recommended to be used to maintain health and to combat certain ailments, and thus several herbal food supplements are now available in the market. Our study suggests that *C. edulis* can be added to the list of herbal supplement formulations to serve as antioxidant and nutraceuticals and to meet increasing demands of the community for such food

Table 5
Binding free energy and inhibition constants of compounds against AChE, BChE, α-amylase, α-glucosidase and Tyrosinase.

Compound	α-glucosidase		α-amylase		AChE		BChE		Tyrosinase	
	Binding free energy (kcal/mol)	Inhibition constants	Binding free energy (kcal/mol)	Inhibition constants	Binding free energy (kcal/mol)	Inhibition constants	Binding free energy (kcal/mol)	Inhibition constants	Binding free energy (kcal/mol)	Inhibition constants
*Reference Compounds	−10.85	11.08 nM	−11.03	8.16 nM	−9.27	161.15 nM	−8.11	1.14 μM	−4.33	666.36 μM
Alliospiroside C	−8.24	919.19 nM	−9.2	178.96 nM	−9.53	103.80 nM	−12.66	524.57 pM	−6.75	11.28 μM
β-Solanine	−8.74	389.9 nM	−10.28	28.98 nM	−11.55	3.43 nM	−11.16	6.59 nM	−7.01	7.31 μM
Cucurbitacin A	−8.52	565.07 nM	−8.14	1.08 μM	−10.61	16.76 nM	−10.23	31.95 nM	−7.77	2 μM
Cucurbitacin L	−8.6	495.71 nM	−8.68	431.7 nM	−9.98	48.40 nM	−11.03	8.28 nM	−7.8	1.91 μM
(3S,7E,9S)-9-Hydroxy-4,7-megastigmadien-3-one 9-glucoside	−8.06	1.24 μM	−7.11	6.12 μM	−10.09	40.00 nM	−9.98	48.53 nM	−6.9	8.75 μM
Lycoperoside D	−10.13	37.57 nM	−10.17	35.25 nM	−8.49	595.98 nM	−11.4	4.42 nM	−5.48	95.89 μM
5,7-Megastigmadien-9-ol glucoside	−7.56	2.87 μM	−7.03	6.97 μM	−9.47	114.16 nM	−9.47	114.16 nM	−6.51	16.95 μM
21-Acetoxy-11b,17-dihydroxy-6?-methylpregn-4-ene-3,20-dione	−8.55	540.09 nM	−8.3	828.74 nM	−10.77	12.84 nM	−10.77	12.84 nM	−7.36	4.03 μM
Momordicoside B	−5.04	202.59 μM	−5.99	40.88 μM	−3.58	2.39 mM	−11.00	8.67 nM	−2.4	17.54 mM
Momordicoside E	−7.99	1.4 μM	−8.2	973.27 nM	−11.82	2.17 nM	−12.06	1.44 nM	−6.77	10.81 μM

Table 6
Details of interaction patterns after post-dock analysis of docked complex for selected compounds.

Ligands	Bond Category	*Bond Distance*	*Bond type*	*Interactions*			
				Residue Name and Groups	*From* Chemistry	*Residue Name and Groups*	To Chemistry
Interaction Patterns for α-amylase							
β-Solanine	Hydrogen Bond	1.67896	Conventional Hydrogen	:UNN0:H	H-Donor	A:ILE152:O	H-Acceptor
		1.75992	Bond	:UNN0:H		A:ILE152:O	
		2.21359		:UNN0:H		A:TYR155:OH	
		1.73812		:UNN0:H		:UNN0:O	
	Hydrogen Bond	3.37984	Carbon Hydrogen Bond	:UNN0:C	H-Donor	A:GLY167:O	H-Acceptor
	Hydrophobic	3.52111	Pi-Sigma	:UNN0:C	C-H	A:HIS80	Pi-Orbitals
		3.99856		:UNN0:C		A:TYR155	
	Hydrophobic	5.10446	Alkyl	A:LEU166	Alkyl	:UNN0	Alkyl
		4.4008		A:LEU166		:UNN0	
		5.26813		:UNN0:C		A:LEU166	
	Hydrophobic	4.35319	Pi-Alkyl	A:TYR75	Pi-Orbitals	:UNN0:C	Alkyl
		4.84736		A:HIS80		:UNN0	
		4.27832		A:HIS80		:UNN0	
		5.09079		A:TYR82		:UNN0	
		4.63054		A:TYR82		:UNN0:C	
		4.29248		A:TYR82		:UNN0:C	
		4.71749		A:TRP83		:UNN0	
		5.24052		A:TRP83		:UNN0	
		5.02656		A:TRP83		:UNN0:C	
		5.35386		A:HIS122		:UNN0:C	
Lycoperoside D	Hydrogen Bond	2.778	Conventional Hydrogen	A:LEU232:HN	H-Donor	:UNN0:O	H-Acceptor
		1.79775	Bond	:UNN0:H		:UNN0:O	
		2.27031		:UNN0:H		A:LEU232:O	
	Hydrogen Bond	3.09624	Carbon Hydrogen Bond	:UNN0:C	H-Donor	A:ASP340:OD2	H-Acceptor
	Hydrophobic	3.8547	Pi-Sigma	:UNN0:C	C-H	A:HIS80	Pi-Orbitals
	Hydrophobic	4.3964	Alkyl	A:LEU166	Alkyl	:UNN0	Alkyl
	Hydrophobic	5.20099	Pi-Alkyl	A:TYR75	Pi-Orbitals	:UNN0	Alkyl
		5.25829		A:HIS80		:UNN0	
		4.32784		A:HIS80		:UNN0:C	
		5.28361		A:TYR82		:UNN0	
		5.10581		A:TYR82		:UNN0:C	
		4.53941		A:TRP83		:UNN0	
		4.76375		A:TRP83		:UNN0	
		4.08468		A:HIS122		:UNN0:C	
Interaction Patterns for α-glucosidase							
β-Solanine	Hydrogen Bond	2.22342	Conventional Hydrogen	A:ALA234:HN	H-Donor	:UNN0:O	H-Acceptor
		2.11332	Bond	:UNN0:H		A:LYS506:O	
		2.43108		:UNN0:H		A:ASP232:O	
		1.79747		:UNN0:H		A:ASP232:O	
		2.11532		:UNN0:H		:UNN0:O	
	Hydrogen Bond	2.77081	Carbon Hydrogen Bond	A:ILE233:CA	H-Donor	:UNN0:O	H-Acceptor
		3.60276		A:PHE476:CA		:UNN0:O	
		3.31517		:UNN0:C		A:ASP630:OD2	
		3.61119		:UNN0:C		A:ASP630:OD1	
	Hydrophobic	3.74241	Pi-Sigma	:UNN0:C	C-H	A:PHE476	Pi-Orbitals
	Hydrophobic	4.12878	Alkyl	A:ALA602	Alkyl	:UNN0	Alkyl
		5.44764		A:ALA628		:UNN0	
		3.07727		A:ALA628		:UNN0:C	
	Hydrophobic	5.26108	Pi-Alkyl	A:TRP329	Pi-Orbitals	:UNN0	Alkyl
		4.90642		A:TRP329		:UNN0:C	
		4.52453		A:TRP329		:UNN0:C	
		4.01679		A:TRP432		:UNN0:C	
		4.61759		A:PHE476		:UNN0	
		4.505		A:PHE601		:UNN0:C	
Lycoperoside D	Hydrogen Bond	2.89744	Conventional Hydrogen	A:ALA234:HN	H-Donor	:UNN0:O	H-Acceptor
		2.42686	Bond	A:ASN475:HN		:UNN0:O	
		2.29353		A:LYS506:HZ3		:UNN0:O	
		2.18926		:UNN0:H		A:ASP630:OD2	
		2.66266		:UNN0:H		A:ASP232:OD2	
		1.84786		:UNN0:H		A:SER474:OG	
		2.26489		:UNN0:H		A:ASP232:O	
		1.65344		:UNN0:H		:UNN0:O	
	Hydrogen Bond	3.53454	Carbon Hydrogen Bond	:UNN0:C	H-Donor	A:ASP232:O	H-Acceptor
	Hydrophobic	4.97282	Alkyl	A:ALA602	Alkyl	:UNN0	Alkyl
		3.05881		A:ALA602		:UNN0:C	
	Hydrophobic	4.95858	Pi-Alkyl	A:PHE236	Pi-Orbitals	:UNN0:C	Alkyl
		5.35584		A:TRP329		:UNN0	
		5.16252		A:TRP432		:UNN0	
		5.10164		A:PHE601		:UNN0	
		5.49935		A:PHE601		:UNN0:C	

(*continued on next page*)

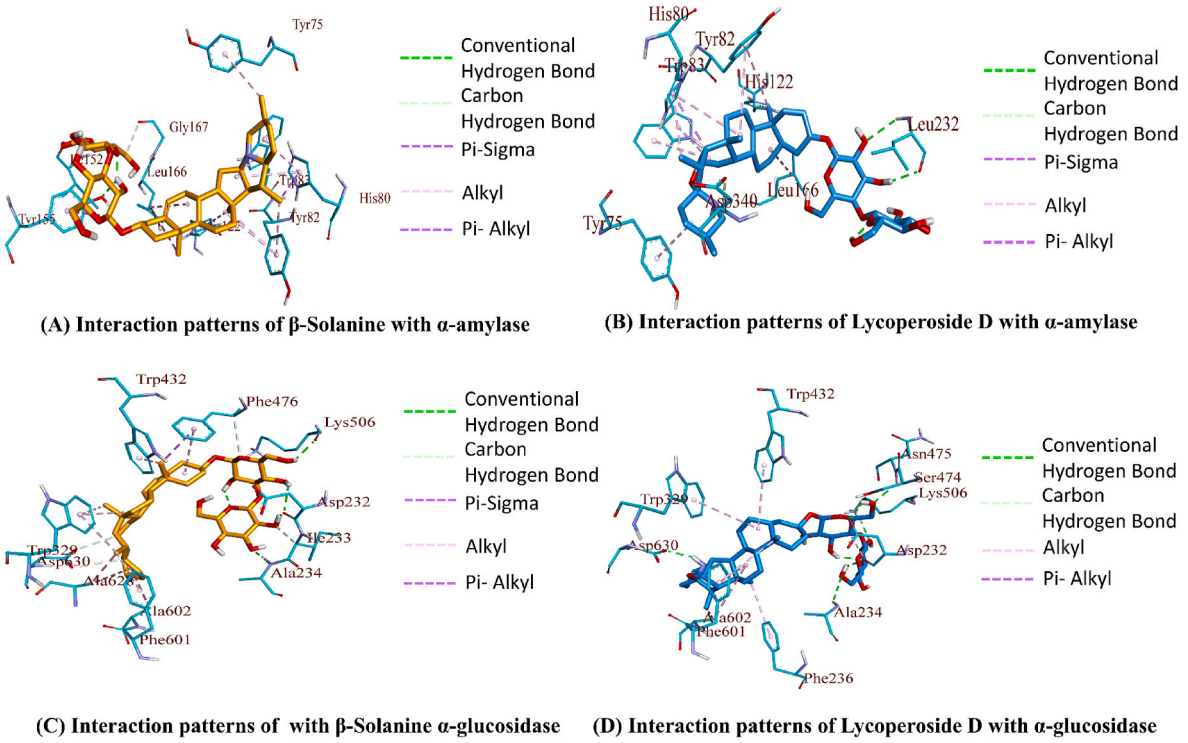
Table 6 (*continued*)

Ligands	Bond Category	*Bond Distance*	*Bond type*	*Interactions*			
				Residue Name and Groups	*From* Chemistry	*Residue Name and Groups*	To Chemistry
Interaction Patterns for AChE							
β-Solanine	Hydrogen Bond	2.09152	Conventional Hydrogen	A:TYR124:HH	H-Donor	:UNN0:O	H-Acceptor
		1.71607	Bond	A:SER125:HG		:UNN0:O	
		1.97222		A:TYR337:HH		:UNN0:O	
		2.6072		A:HIS447:HE2		:UNN0:O	
		2.706		:UNN0:H		A:ASN87:OD1	
		1.81182		:UNN0:H		:UNN0:O	
	Hydrogen Bond	2.8054	Carbon Hydrogen Bond	:UNN0:C	H-Donor	A:TYR124:OH	H-Acceptor
	Hydrophobic	4.70416	Alkyl	A:LEU289	Alkyl	:UNN0	Alkyl
		4.07799		:UNN0:C		A:LEU76	
		4.49703		:UNN0:C		A:LEU289	
	Hydrophobic	5.29454	Pi-Alkyl	A:TYR72	Pi-Orbitals	:UNN0	Alkyl
		5.04588		A:TRP286		:UNN0	
		4.34809		A:TRP286		:UNN0	
		5.07145		A:TRP286		:UNN0	
		4.85098		A:TRP286		:UNN0	
		4.88223		A:TYR341		:UNN0	
		4.22339		A:TYR341		:UNN0:C	
Momordicoside E	Hydrogen Bond	2.96832	Conventional Hydrogen	A:TYR337:HH	H-Donor	:UNN0:O	H-Acceptor
		1.63616	Bond	:UNN0:H		:UNN0:O	
	Hydrogen Bond	3.53111	Carbon Hydrogen Bond	A:GLY121:CA	H-Donor	:UNN0:O	H-Acceptor
		3.70402		:UNN0:C		A:ASP74:OD1	
	Hydrophobic	3.85656	Pi-Sigma	:UNN0:C	C-H	A:TRP286	Pi-Orbitals
		3.65699		:UNN0:C		A:TRP86	
	Hydrophobic	4.46838	Pi-Alkyl	A:TYR72	Pi-Orbitals	:UNN0:C	Alkyl
		4.7503		A:TRP286		:UNN0	
		4.26458		A:TRP286		:UNN0	
		3.90704		A:TRP286		:UNN0	
		4.18961		A:TRP286		:UNN0:C	
		4.19287		A:TRP286		:UNN0	
		4.70991		A:TYR341		:UNN0:C	
Interaction Patterns for BChE							
Alliospiroside C	Hydrogen Bond	2.48119	Conventional Hydrogen	A:THR120:HG1	H-Donor	:UNN0:O	H-Acceptor
		2.76295	Bond	A:TYR128:HH		:UNN0:O	
		2.01891		A:SER287:HG		:UNN0:O	
		1.94952		:UNN0:H		A:GLU197:OE1	
		2.13458		:UNN0:H		A:GLU197:OE1	
		2.09713		:UNN0:H		A:ASN83:OD1	
		2.07703		:UNN0:H		A:TRP82:O	
		2.9233		:UNN0:H		A:GLY115:O	
	Hydrogen Bond	2.86103	Carbon Hydrogen Bond	A:GLY439:CA	H-Donor	:UNN0:O	H-Acceptor
		3.47387		:UNN0:C		A:LEU286:O	
		3.52307		:UNN0:C		A:HIS438:O	
	Hydrophobic	3.73328	Pi-Sigma	:UNN0:C	C-H	A:TRP82	Pi-Orbitals
	Hydrophobic	4.70234	Alkyl	A:ALA328	Alkyl	:UNN0	Alkyl
		5.24629		A:TRP82		:UNN0	
		5.20792		A:TRP82		:UNN0:C	
		4.65749		A:PHE329		:UNN0	
		4.13196		A:TYR332		:UNN0	
Momordicoside E	Hydrogen Bond	2.3722	Conventional Hydrogen	A:HIS438:HE2	H-Donor	:UNN0:O	H-Acceptor
		2.04442	Bond	:UNN0:H		A:PRO285:O	
		2.04232		:UNN0:H		A:PRO285:O	
		2.57403		:UNN0:H		A:LEU286:O	
		2.74255		:UNN0:H		A:GLY78:O	
	Hydrogen Bond	3.204	Carbon Hydrogen Bond	:UNN0:C	H-Donor	:UNN0:O	H-Acceptor
	Hydrophobic	3.93425	Pi-Sigma	:UNN0:C	C-H	A:TYR332	Pi-Orbitals
Interaction Patterns for Tyrosinase							
Cucurbitacin A	Hydrogen Bond	2.12362	Conventional Hydrogen	A:HIS60:HD1	H-Donor	:UNN0:O	H-Acceptor
		1.97188	Bond	A:VAL218:HN		:UNN0:O	
		2.61866		:UNN0:H		:UNN0:O	
		1.81388		:UNN0:H		A:GLY216:O	
		1.7138		:UNN0:H		A:GLU158:OE2	
	Hydrogen Bond	3.11713	Carbon Hydrogen Bond	A:HIS204:CE1	H-Donor	:UNN0:O	H-Acceptor
	Hydrophobic	3.65555	Pi-Sigma	:UNN0:C	C-H	A:HIS208	Pi-Orbitals
	Hydrophobic	3.28011	Alkyl	A:CU501:CU	Alkyl	:UNN0:C	Alkyl
		4.46707		A:CU502:CU		:UNN0:C	
		4.58213		:UNN0:C		A:VAL217	
		4.15693		:UNN0:C		A:VAL218	
	Hydrophobic	4.24755	Pi-Alkyl	A:HIS60	Pi-Orbitals	:UNN0:C	Alkyl
		5.21891		A:PHE197		:UNN0	
		5.12551		A:HIS204		:UNN0:C	
		4.26436		A:HIS204		:UNN0:C	
Cucurbitacin L	Hydrogen Bond	2.13959		A:VAL218:HN	H-Donor	:UNN0:O	H-Acceptor

(*continued on next page*)

Table 6 (*continued*)

Ligands	Bond Category	*Bond Distance*	*Bond type*	*Interactions*			
				Residue Name and Groups	*From* Chemistry	*Residue Name and Groups*	To Chemistry
		2.32334	Conventional Hydrogen	:UNN0:H		A:MET215:O	
		1.79993	Bond	:UNN0:H		A:GLU158:OE2	
	Hydrogen Bond	3.01874	Pi-Donor Hydrogen Bond	:UNN0:H	H-Donor	A:HIS208	Pi-Orbitals
	Hydrophobic	3.2502	Pi-Sigma	:UNN0:C	C-H	A:HIS208	Pi-Orbitals
	Hydrophobic	4.51332	Alkyl	A:PRO201	Alkyl	:UNN0	Alkyl
		3.9289		A:CU501:Cu		:UNN0:C	
		4.28993		A:CU502:Cu		:UNN0:C	
		4.9903		:UNN0:C		A:VAL218	
	Hydrophobic	5.25758	Pi-Alkyl	A:PHE197	Pi-Orbitals	:UNN0	Alkyl
		5.23447		A:HIS204		:UNN0:C	



(A) Interaction patterns of β-Solanine with α-amylase **(B) Interaction patterns of Lycoperoside D with α-amylase**

(C) Interaction patterns of with β-Solanine α-glucosidase **(D) Interaction patterns of Lycoperoside D with α-glucosidase**

Fig. 1. Diagrammatic representation of β-Solanine and Lycoperoside D with active sites of α-amylase and α-glucosidase.

supplements and formulations. Since *C. edulis* is already being used as emergency food by the local community, toxicity threats are ruled out, which finally make this non-conventional food plant a strong candidate to be commercially used for disease prevention and health promoting benefits.

3.4. Enzyme inhibition activities

Cholinesterases are responsible to develop neurodegenerative disorders, which impair normal functioning of brain, especially in old age. The cholinergic system (organized nerve cells) is triggered during the nerve impulse and release the neurotransmitter acetylcholine, which is broken down into acetic acid and choline. Therefore, inhibiting the activity of the enzymes cholinestrases decreases the breakdown of the acetylcholine, and in turn, the increased concentration of acetylcholine in the brain improves communication between the brain nerve cells thus decreasing the Dementia and Alzheimer's disease. Therefore, AChE and BChE inhibitors have become standard approach in treatment of neurodegenerative diseases (Zengin et al., 2019). Keeping in view these ideas, the extracts of *C. edulis* were evaluated against the enzymes AChE and BChE. The extracts exhibited significant inhibition of both the cholinestrases (Table 4). Ce-E showed highest activity against AChE (2.37 ± 0.04 mg GALAE/g extract) followed by Ce-H (2.04 ± 0.30 mg GALAE/g extract) and Ce-M (1.94 ± 0.09 mg GALAE/g extract), however Ce-W was inactive. In BChE assay, Ce-H exhibited the highest (6.43 ± 0.95 mg GALAE/g extract) inhibition, Ce-M showed the least potential with a value of 3.12 ± 0.50 mg GALAE/g extract, whereas, Ce-W was inactive (Table 4). Previous studies substantiate our results, which indicate that among acetone and chloroform extracts, the low polar extracts showed higher AChE and BChE inhibitory potential, however, these studies further disclosed that such fractions contain cinnamic acid, ferulic and methoxycinnamic acid isomers (Zengin et al., 2018). Several other studies carried out during the current decade predicted that anti-Alzheimer's activity of plant extracts is attribute to phenolics and flavonoids targeting cholinesterases in silico (Das et al., 2017; Işık and Beydemir, 2021; Khan et al., 2009; Zhou et al., 2015) and have established a correlation between phenolics and flavonoids and anti-cholinestarse activity, Thus, in the present study, the highest cholinestrase inhibitory activity of Ce-E and Ce-H could be attributed to the presence of metabolites like cinnamic acid, its derivatives and high contents of phenolics and flavonoids in these fractions.

In addition, the extracts were also studied *in vitro* for their inhibitory activity against α-glucosidase and α-amylase, the enzymes responsible for diabetes mellitus, which is now a global challenge to be addressed.

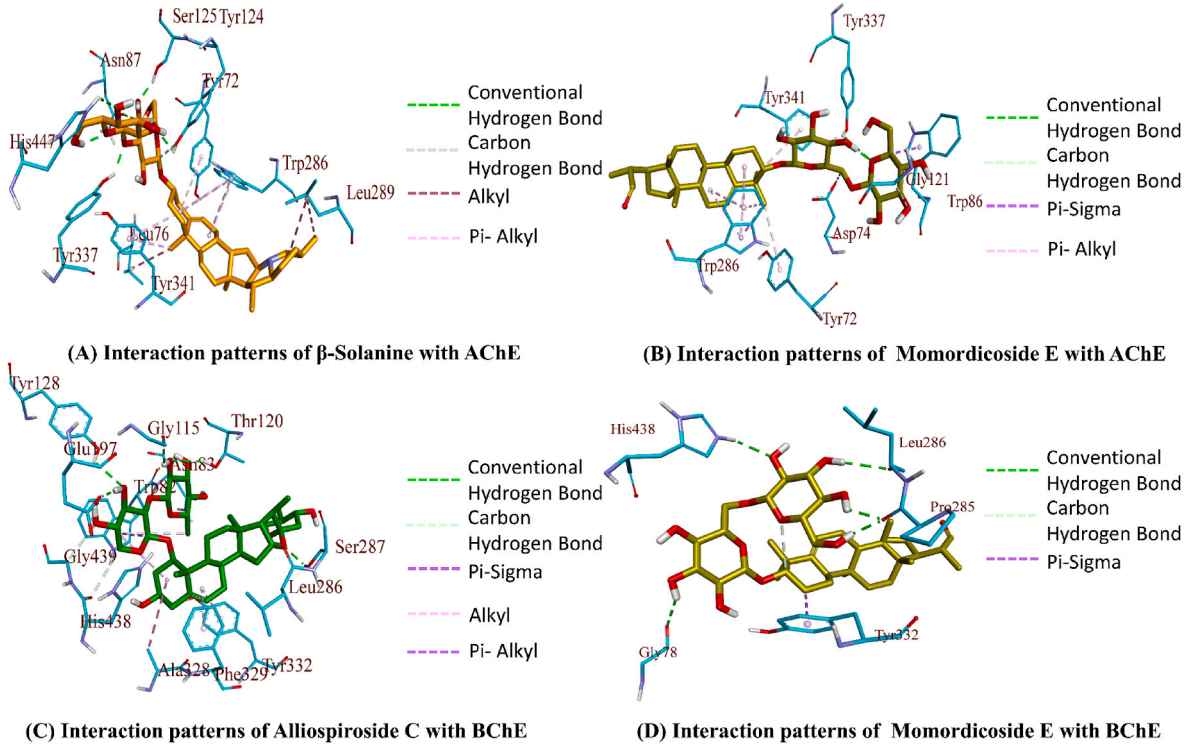


Fig. 2. Diagrammatic representation of β-Solanine, Momordicoside E and Alliospiroside C with active sites of AChE and BChE.

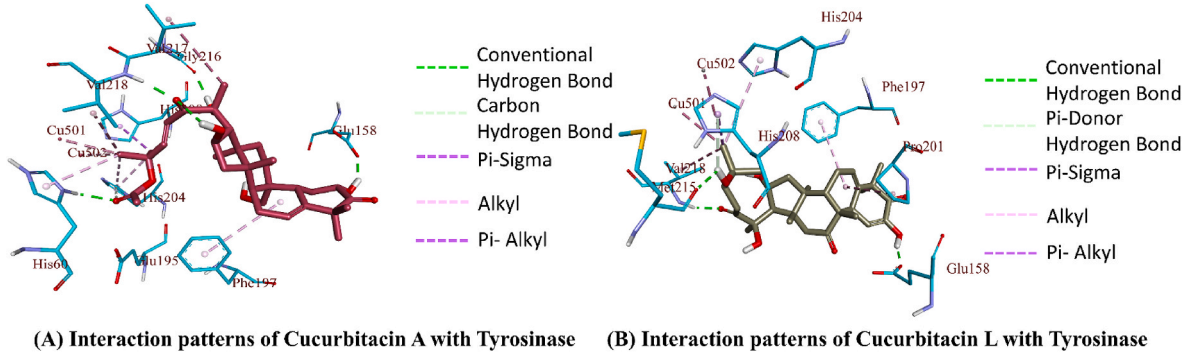


Fig. 3. Diagrammatic representation of Cucurbitacin A and Cucurbitacin L with active sites of Tyrosinase.

Dependence on insulin and synthetic drugs invites other diseases as well. It is shown that the inhibition of the enzymes α-amylase and α-glucosidase which are responsible for carbohydrate breakdown in glucose can control diabetic problems. α-amylase hydrolyze starch into glucose and its inhibition can reduce post prandial hyperglycemia (Chelladurai and Chinnachamy, 2018). Among the tested fractions, the Ce-H was most effective against α-amylase (0.47 ± 0.01 mmol ACAE/g extract) and α-glucosidase (6.98 ± 1.06 mmol ACAE/g) enzymes, while Ce-E was moderately active against α -glucosidase with value of 4.21 ± 0.55 mmol ACAE/g of the extract. Interestingly, Ce-M was observed inactive towards α glucosidase; the reason could be that active compounds concentration is suppressed in Ce-M. Ce-W remained inactive against both the enzymes (Table 4). These results substantiate the right use of *Caralluma* plants as antidiabetic agent and thus *C. edulis* can also be used as antidiabetic plant. Cosmetic industry requires developing and producing skin care and whitening products, which minimize skin pigmentation. These products work by limiting or inhibition of a copper-containing enzyme tyrosinase, which catalyzes synthesis of melanin in human skin. The excessive production of melanin causes melasma, freckles, and Parkinson disease. Thus there is crucial need to search for safer tyrosinase inhibitors, which are important in controlling melanin biosynthesis in mammals, microorganisms, fungi and plants thus have potential applications in cosmetics, pharmaceutical and food industry (Şöhretoğlu et al., 2018). Keeping in view the continual need to search for novel and safe tyrosinase inhibitors of natural origin (Panzella and Napolitano, 2019; Shi et al., 2020), the extracts of *C. edulis* were evaluated *in vitro* against the activity of the enzyme tyrosinase. The results disclosed the Ce-H fraction as significant inhibitor with the value of 64.46 ± 0.69 mg KAE/g of the extract (Table 4). Ce-M and Ce-E were also equally active (59.85 ± 0.42 and 58.40 ± 0.38 mg KAE/g extract), While Ce-W exhibited was recorded as least tyrosinase inhibitor (3.19 ± 0.93 mg KAE/g extract). Literature report clearly mentioned that aqueous plant extracts are poor inhibitors of the enzyme tyrosinase, while methanol and ethyl acetate extracts exhibited significant activities (Haliloglu et al., 2017). Our results are also substantiated by other research reports published by Zhang et al. that various plant extracts except water extract show potent tyrosinase inhibitory activities (Zhang et al., 2022). These results proposed the possible use of *C. edulis* extracts in skin whitening and anti-browning formulations.

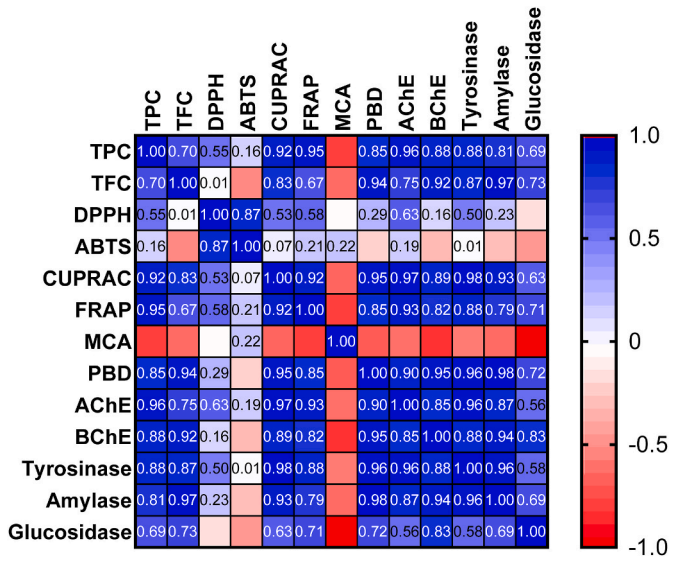


Fig. 4. Pearson correlation values between biological activity assays ($p < 0.05$). TPC: Total phenolic content; TFC: Total flavonoid content; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; DPPH: 1,1-diphenyl-2-picrylhydrazyl; CUPRAC: Cupric reducing antioxidant capacity; FRAP: Ferric reducing antioxidant power; MCA: Metal chelating ability; PBD: Phosphomolybdenum; AChE: acetylcholinesterase; BChE: butyrylcholinesterase.

3.5. Post dock analysis

A powerful and capable tool for in silico screening is molecular docking. It is becoming ever more significant in the process of rational drug design (Meng et al., 2011). Finding a suitable ligand that interacts with protein's binding site energetically and geometrically is a computational based process called docking (Kaapro and Ojanen, 2002). The best binding pose for each compound was identified on the basis of rmsd clustering having highest binding free energy in terms of negative values for 250 runs out of each docking with the selected compounds. An overview of the outcomes for docking studies with the five enzymatic proteins is shown in Tables 5 and 6. The docking interaction of best dock compounds, β-solanine and lycoperoside D with crystallographic α-amylase and α-glucosidase are shown in Fig. 1. (A, B, C and D). Similarly, the docking interaction of best dock compounds (β-solanine and momordicoside E) with crystallographic AChE is shown in Fig. 2 (A and B). The interaction patterns for alliospiroside C-BChE complex and momordicoside E-BChE complex are shown in Fig. 3 (C and D). While in case of tyrosinase Fig. 3 (A and B) represents interaction with cucurbitacin A and cucurbitacin L. The binding affinities for α-amylase ranged from −5.99 to −10.28 kcal/mol in comparison to acarbose (10.83 kcal/mol, Table 5). The β-solanine, which has a value of −10.28 kcal/mol, was discovered to be the best among all selected compounds against α-amylase while lycoperoside D has binding affinity −10.13 kcal/mol against α-glucosidase and has showed highest binding affinities than rest of the compounds. The momordicoside E showed highest binding affinities −11.82, and −12.06 kcal/mol for AChE and BChE respectively. The binding free energy and inhibition constants for docked complexes is given in Table 5. The top two docked complexes for the five enzymatic proteins were studied for interaction patterns via Discovery Studio Visualizer. According to a thorough investigation at the binding site of α-amylase β-solanine contains four hydrogen bonds, a carbon hydrogen bond and three different types of hydrophobic interactions, while β-solanine formed three hydrogen bonds along with carbon hydrogen bond and three different types of hydrophobic interactions. The lycoperoside D and β-solanine formed hydrogen bonds, carbon hydrogen bond and hydrophobic interactions with α-glucosidase while β-solanine mediated an additional hydrophobic interaction (Fig. 1 C and D). The binding interaction patterns for AChE given in Fig. 2 A-B showed that β-solanine and momordicoside E exhibited conventional hydrogen bonds and some hydrophobic interactions while alliospiroside and momordicside E mediated similar interactions for BChE (Fig. 2. C and D). The cucurbitacin A and cucurbitacin L outperformed against tyrosinase and mediated similar hydrogen bonds and hydrophobic interactions with binding affinities −7.77 and −7.8 kcal/mol respectively (Fig. 3. A and B). The details for interactions of top two complexes for each of the five enzymes is provided in Table 6.

3.6. Multivariate analysis

In the present study, we performed a multivariate analysis, namely Pearson's correlation and principal component analysis (PCA), with the results of total bioactive components, antioxidant properties and enzyme inhibitory effects. The results are shown in Figs. 4 and 5. In Pearson's correlation, the antioxidant capacity assays, with the exception of the scavenger and metal chelate assays, correlated strongly with total phenol content. In particular, the reducing power assays showed a good correlation ($R > 1$) with the total phenolic content of the tested extracts (Fig. 4). In consistent with our results, several researchers were reported that a linear correlation between total phenolic content and antioxidant properties of plant extracts (ben Jalloul et al., 2022; Vo

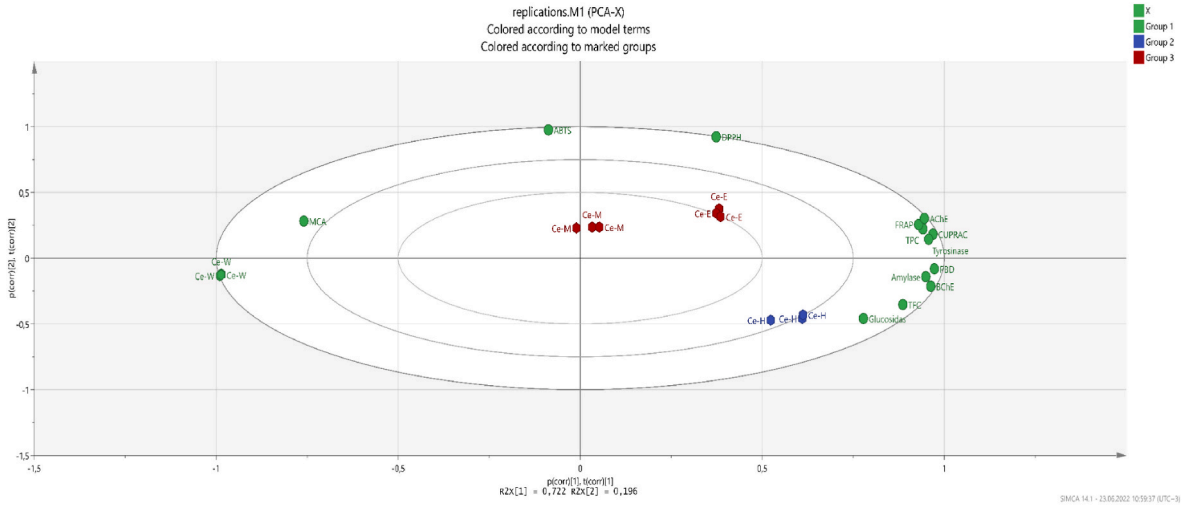


Fig. 5. Biplot graph from principal component analysis between *C. edulis* extracts and biological activities.

et al., 2022). Interestingly, the correlation between the total phenolic and radical scavenging assays was weak ($R < 0.5$). This finding could be explained with the possible presence of non-phenolic antioxidants like vitamin C or tocopherol in the extracts and this approach was also supported by some authors (Ghimire et al., 2021; Sulaiman et al., 2011). However, the total phenolic content was strongly associated with the enzyme inhibitory properties. Several phenolic compounds have been reported as potent enzyme inhibitors agents in recent studies (John et al., 2021; Mukherjee et al., 2018; Papoutsis et al., 2021), and at this point the tested extracts have great potential as sources of natural enzyme inhibitors. In addition to the correlation analysis, the PCA analysis (two components: PC1: 72.2%; PC2:19.65) reflected that the tested extracts were divided into three groups (Fig. 5). Methanol and ethyl acetate extracts were placed in same group and had similar actions in the biological activity assays. Other extracts, namely n-hexane and water were in other groups. In fact, the solvents were distributed based on their polarities. Since ethyl acetate and methanol have close polarity, they were in the same group. The result showed that the selection of the extraction solvents is an important step and therefore our results could be useful for the production of functional ingredients using *C. edulis*.

4. Conclusion

In the present work, we studied different extracts of *C. edulis* for their *in vitro* antioxidant, and *in vitro* and in silico enzyme inhibition properties. The ethyl acetate fraction of the methanolic extract contains highest phenolic and flavonoid contents and also potentially inhibited the activity of α-amylase and α-glucosidase, AChE and BChE and tyrosinase. The same extract also exhibited highest metal reducing power and radical scavenging property and thus has been declared as potential antioxidant herbal source. The crude methanolic extract was subjected to UHPLC-MS analysis and was found rich in diverse secondary metabolites; these findings authenticate the strong antioxidant and enzyme inhibition potential of *C. edulis* and thus can be a potential candidate for commercial applications in various formulations. Moreover, the herbal extracts which are rich in phenolics and steroids or their glycosides are used as nutraceuticals in several health formulations. *Tribulus terrestris* (Abdel-All et al., 2021; Chhatre et al., 2014; Santos et al., 2019) and *Panax ginseng* (Endale et al., 2014; Kim, 2016; Kimura et al., 2006; Riaz et al., 2019) are common references in this regard, which are being used as nutraceuticals in several food supplements with their flavonoid and steroid glycosides as their active components. Ethyl acetate extract of *C. edulis* has also been found rich in flavonoid and probably the steroid glycosides; therefore, it also offers its candidature as potential nutraceutical or food supplement ingredient. The present findings offer preliminary data for the valorization of *C. edulis* as a source of bioactive constituents with potential use in health formulations to prevention and treat various human diseases.

CRediT authorship contribution statement

Natasha Shazmeen: Conceptualization, Visualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing – Original Draft. Mamona Nazir: Conceptualization, Resources, Investigation, Writing – Review & Editing. Naheed Riaz: Conceptualization, Resources, Investigation, Writing – Review & Editing. Muhammad Saleem: Resources, Investigation, Writing – Review & Editing. Muhammad Imran Tousif: Resources, Investigation, Writing – Review & Editing. Saba Tauseef: Formal analysis, Software, Writing – Original Draft. Reaz Uddin: Formal analysis, Software, Writing – Original Draft. Mahreen Mukhtar: Formal analysis, Software, Writing – Original Draft. Gokhan Zengin: Writing – Review & Editing, Supervision. Adriano Mollica: Writing – Review & Editing, Supervision. Abdul aziz A. Zarhah: Writing – Review & Editing, Supervision. Saleh S. Alarfaji: Writing – Review & Editing, Supervision. Shabbir Muhammad: Writing – Review & Editing, Supervision.

Data availability

No data was used for the research described in the article.

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