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PHYTOCHEMICAL AND IN VITRO ANTIOXIDANT ACTIVITY OF *ZIZANIA AQUATICA*

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ABSTRACT
The antioxidant capacity of *Zizania aquatica*.'s ethanolic extract was assessed using *in vitro* studies. The extract efficacy was assessed using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH), reducing power, nitric oxide, hydrogen peroxide and superoxide dismutase (SOD) assays. The outcomes of every technique demonstrated that Z. aquatica's ethanolic extract has the ability to function as an antioxidant in a concentration-dependent manner. Ascorbic acid was used as a benchmark to evaluate the sample's antioxidant activity to. The ethanolic extract of Z. aquatica exhibited prominent antioxidant potential in all assays, positioning it as a promising source of natural antioxidants.

KEYWORDS: *Zizania aquatica, phytochemistry, antioxidant, Gas chromatography-Mass spectroscopy.*

INTRODUCTION

The earliest known medical practice is herbal therapy. Throughout history, all cultures have employed herbs. About 80% of people on the planet already utilize herbal medicine for some form of primary healthcare, according to estimates from the World Health Organization (WHO). Ayurvedic, homeopathic, naturopathic, traditional Oriental, and Native American Indian medicine heavily rely on herbal treatments. According to the WHO, out of 119 pharmaceuticals produced from plants, over 74% are utilized in contemporary medicine in ways that are directly related to their traditional applications as plant medicines by indigenous societies. In order to determine whether plant materials collected from rain forests and various regions to explore their potential medical use.

Due to the limitations of allopathic medications to treat stress-related environmental and psychological illnesses, a history of surgical mishaps, and the development of antibiotic resistance, individuals are increasingly seeking alternative medical treatments. Additionally, certain chronic conditions include cancer, immunodeficiency, migraines, irritable bowel syndrome, asthma, arthritis, insomnia, jaundice, and immunodeficiency lack a viable conventional treatment. A number of patients with acquired immunodeficiency syndrome (AIDS) or cancer are presently incorporating one or more alternative therapies into their treatment regimens. The unfavorable impacts of traditional medicine are also prompting individuals to explore alternative medical practices. In Asia, the scientific community recognizes the Zizania aquatica species of black rice, a member of the Oryza sativa L. family for its potential health benefits.

Around the world, there are 200 different types of black rice. China produces 62% of the black rice produced worldwide. China has generated about 45 contemporary black rice varieties with numerous resistance genes and high yields (Sompong *et al.*, 2011). Compared to brown and white rice, black rice is rich in dietary fiber and minerals such as calcium (Ca), phosphorous (P), iron (Fe), and zinc (Zn). The phytochemical composition of black rice is remarkable, encompassing essential and plentiful compounds. Twenty-three secondary metabolites, including anthocyanins, flavones, isorhamnetin-3-O-glucoside, and myricetin-7-O-glucoside, as well as flavonoid glycosides, quercetin-3-O-glucoside, vitamin E (tocopherols and tocotrienols), carotenoids, and y-oryzanol, have been qualitatively and quantitatively characterized and are contribute to the health-promoting properties of black rice. Around the world, there are 200 different types of black rice. Additionally, the black rice is transformed into a functional food (Sriseadka *et al.*, 2012, Irakli *et al.*, 2012). The distinctive deep hue of black rice is attributed to anthocyanins with approximately 26.3% of black rice containing these compounds.Cyanidin-3-O-glucoside

and peonidin-3-O-glucoside are the primary active ingredients among anthocyanin's. The investigation pursues to identify Z. aquatica antioxidant characteristics.

MATERIALS AND METHODS

We locally purchased the Z. aquatica in Trichy. The shade dried Z. aquatica was mechanically pulverized and kept in an airtight container. Using a Soxhlet apparatus, the extraction process was performed using the sequential solvent extraction method. Hexane, ethanol, and aqueous extracts of Z. aquatica were prepared using the sequentially using this technique. Following extraction, several phytochemical analyses were conducted. Subsequently, the compounds present in the extract of Z. aquatica were identified through anti-oxidant assays such as hydrogen peroxide scavenging, reducing power (Oyaizu, 1986), nitric oxide scavenging activity (Garrat, 1964), superoxide anion scavenging activity (Nishimiki *et al.,* 1972), and DPPH scavenging assay. Gas chromatography analysis was also performed.

RESULT AND DISCUSSION

The current study unequivocally shows that the ethanolic extract of Z. aquatica contains a variety of phytoconstituents, including flavonoids, steroidal glycosides, tannins, and alkaloids. The aqueous extracts, which are abundant in flavonoids, alkaloids, and tannins. The petroleum ether extracts that includes tannins, carbohydrates, and other substances. These plant's secondary metabolites might be the cause of its several therapeutic benefits.

The ethanolic extract of Z. aquatica's exhibited DPPH radical scavenging activity was demonstrated in Table 2 in a concentration-dependent manner. At 1500 µg/ml of the Z. aquatica fraction, the highest percentage of scavenging activity (77.19%) was observed, whereas the lowest percentage (19.04%) was recorded at 250 µg/ml. The decreasing power of the Z. aquatica extract is displayed in Table 3. At 1500 µg/ml, the maximum percentage of reducing power (90.01%) was observed, while the lowest percentage of rising power (50.97%) was obtained at 250 µg/ml, the lower concentration. The nitric oxide scavenging activity of Z. aquatica is presented in Table 4. As the extract concentration rises, there is a noticeable increase in its nitric oxide scavenging activity. The Z. aquatica extract concentration of 1500 µg/ml showed the maximum activity. The hydrogen peroxide scavenging activity is displayed in Table 5. As the concentration of the Z. aquatica extract rises, indicating an increase in the scavenging activity. At 1500 µg/ml, the highest activity was measured, and at 250 µg/ml, the lowest activity. Table 6 shows the results of SOD assay, revealing a higher % of inhibition of the *Z.* aquatica extract at 1500 µg/ml (75.19%) and a lower % of inhibition at 250 µg/ml (12.31%).

An essential test for determining the antioxidant activity of plant extracts in an *in vitro* setting is the DPPH scavenging assay. Free radicals like DPPH readily interact with antioxidant substances. The antioxidative substances exhibit the ability to give DPPH an atom of hydrogen and alter its color. Colorimetry is used to quantify color intensity. The inhibition of DPPH is exactly proportional to the rising intensity of color. The current investigation demonstrates that when extract concentration increases, DPPH activity is inhibited. The highest level of inhibition was observed at 1500 µg/ml.

The plant extract's ability for electron transfer is correlated with its reducing power. This assay measures the ability of Fe3+ to transition to Fe2+ (Meir *et al.,* 1995). The extract had concentration-dependent antioxidant activity, according to the data. As per the findings, Z. aquatica can convert Fe3+ into Fe2+ and reduce oxidative damage in the surrounding tissues.

When sodium nitroprusside reacts with oxygen, it produces nitrite, a free radical known as nitric oxide. The plant extracts that directly react with oxygen, nitric oxide, and other nitrogen compounds were used to test the antioxidant activity by preventing the formation of nitrite (Marcocci *et al.,* 1994). This study proves that the extract's optimum inhibitory action against nitric oxide occurs at increasing concentrations.

Because it could be harmful if hydrogen peroxide is transformed into hydroxyl radicals in the cells, it is a significant reactive oxygen species (Gulcin *et al.,* 2003). According to Mathew and Abraham (2006), antioxidant chemicals neutralize H_2O_2 by donating electrons to it. The current work demonstrates how H_2O_2 can suppress hydroxyl radicals in a concentration-dependent way.

An essential enzyme in the antioxidant defense system is superoxide dismutase (Curtis and Mortiz, 1972). SOD mitigates the harmful effect by converting the superoxide anion into hydrogen peroxide. The amount that SOD inhibits superoxide may lessen the harm that occurs to cells. The current investigation demonstrates that the extract's maximum SOD inhibitory activity increases with extract concentration.

GC-MS analysis of ethanolic extract of *Z. aquatic*

The examination of medicinal plants has made extensive use of GC-MS research in recent years. Fatty acid (FA), lipids, alkaloids, volatile essential oils, and non-polar components can all be effectively analyzed with this methodology. By using GC-MS analysis, the current effort looks at the existence of phytoconstituents in the ethanolic extracts of Z. aquatica. Z. aquatica's GC-MS analysis results are displayed in Figure X and Table X. A total of 122 peaks, including propenoic acid, butanediol, furfural, cyclopentanedione, propanone, benzoic acid, birocetam, hexadecanoic acid, tetradecanoic acid, vanillin, tetradecanol, hexadecane, pyrazino,

heneicosane, hexacosane, and octadecanoic acid, were detected in the ethanol extract of Z. aquatica.

Pharmacologically active substances, including levoglucosenone, possess the potential to treat cardiac disease, autoimmune disorders, and cancer. The most common uses of the chemical piracetam are for breath-holding attacks, seizures, dizziness, a learning disorder characterized by trouble reading, and a movement disorder that is frequently brought on by antipsychotic medications. Treatments for fever, spasms, dysmenorrhea, blood clotting, and gastrointestinal distress include the use of vanillin. According to Tharmaraj *et al.* (2015), heicosane prevents inflammation and has anti-atherosclerotic, antibacterial, anti-plasmodial, hypolipidemic, and anti-atherosclerotic properties. It has been observed that hexadecanoic acid exhibits anti-inflammatory, antibacterial, and antioxidant properties.

Table 1: Phytochemical analysis of various extracts of *Z. aquatic.*

S. No.	Phytochemical Constituents	Name of the Test	Ethanol	Aqueous	Petroleum ether
1.	Alkaloids	Mayer's test	+	+	-
2.	Carbohydrate	Molish test	+	+	-
3.	Tannin	Lead acetate test	+	+	+
4.	Terpenoids	Noller test	+	-	-
5.	Steroidal glycosides	Salkowaski test	-	-	-
6.	Quinones	H_2SO_4 test	-	-	-
7.	Anthraquinone	Borntrager's test	-	-	-
8.	Saponin glycosides	Froth test	+	+	+
9.	Flavonoids	Schioda's test	+	+	-
10.	Total protein	Millon's test	+	+	-
11.	Fixed oil	pot test	-	-	-
12.	Phenols	$FeCl_3$ test	+		
13.	Coumarin	NaCl test	-		

+ = Present – = Absent

Table 2: DPPH assay of ethanolic extract of *Z. aquatic.*

Concentration (µg/ml)	% of inhibition of extract	% of inhibition of standard
250	*19.04*	*23.56*
500	*36.20*	*41.98*
750	*55.79*	*61.91*
1000	*67.09*	*79.86*
1500	*77.19*	*86.71*
IC_{50}	*645*	*595*

Table 3: Reducing power assay of ethanolic extract of *Z. aquatic.*

Concentration (µg/ml)	% of inhibition of extract	% of inhibition of standard
250	*50.97*	*56.81*
500	*60.30*	*65.86*
750	*72.91*	*81.68*
1000	*82.49*	*90.11*
1500	*90.01*	*97.23*
IC_{50}	*240*	*220*

Table 4: Nitric oxide assay of ethanolic extract of *Z. aquatic.*

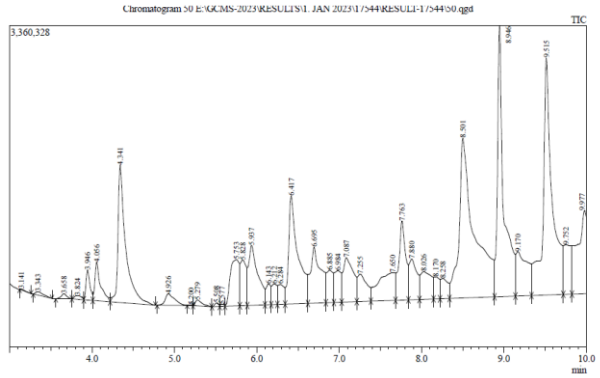
Concentration (µg/ml)	% of inhibition of extract	% of inhibition of standard
250	*21.34*	*28.12*
500	*35.78*	*43.60*
750	*46.91*	*56.81*
1000	*60.22*	*70.16*
1500	*69.78*	*84.19*
IC_{50}	*805*	*630*

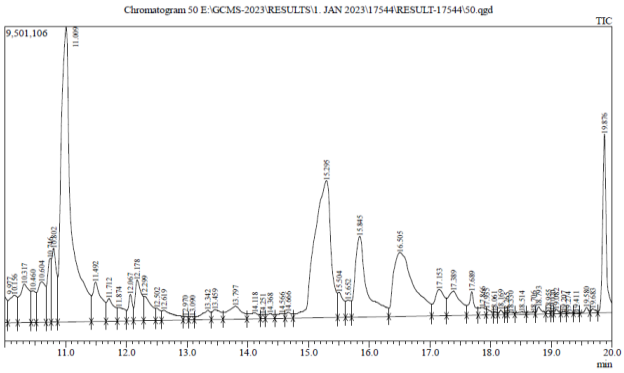
Table 5: H_2O_2 assay of ethanolic extract of *Z. aquatic*.

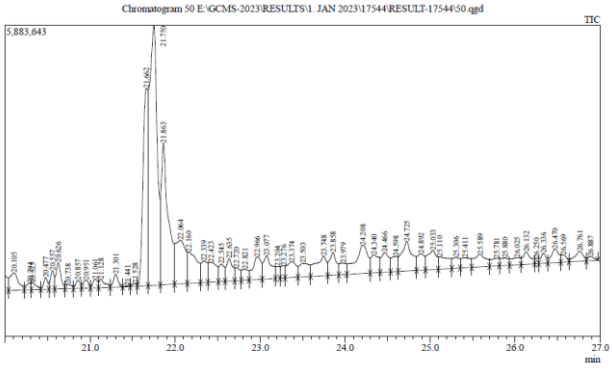
Concentration (µg/ml)	% of inhibition of extract	% of inhibition of standard
250	*19.82*	*29.72*
500	*33.91*	*40.38*
750	*50.17*	*60.16*
1000	*65.31*	*74.31*
1500	*82.16*	*90.81*
IC_{50}	*750*	*670*

Table 6: SOD assay of ethanolic extract of *Z. aquatic*.

Concentration (µg/ml)	% of inhibition of extract	% of inhibition of standard
250	*12.31*	*19.72*
500	*23.27*	*29.19*
750	*45.90*	*50.69*
1000	*60.17*	*64.15*
1500	*75.19*	*81.19*
IC_{50}	*805*	*750*







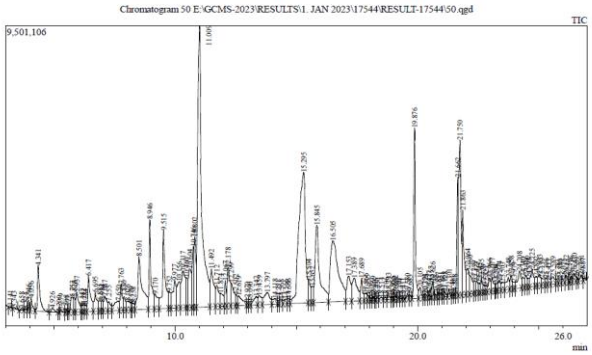


Table 7: The phytoconstituents present in the ethanolic extract of *Z. aquatica*

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height %	A/H Name
1	3.141	3.120	3.260	33893	0.00	10353	0.01	3.27 silanediol, dimethyl-
2	3.343	3.285	3.515	225311	0.03	36161	0.04	6.23 2-propenoic acid
3	3.658	3.560	3.755	246531	0.03	52047	0.06	4.74 1-penten-3-one
4	3.824	3.755	3.900	226720	0.03	39491	0.04	5.74 2-propanone, 1-hydroxy-
5	3.946	3.900	4.010	1051419	0.14	316770	0.36	3.32 2,3-butanediol
6	4.056	4.010	4.220	1918156	0.26	403469	0.45	4.75 2,3-butanediol
7	4.341	4.220	4.770	9311143	1.26	1440428	1.62	6.46 furfural
8	4.926	4.790	5.225	987887	0.13	121537	0.14	8.13 2-furanmethanol
9	5.200	5.155	5.225	4998	0.00	2666	0.00	1.87 trans-5-hydroxy-2-methyl-1,3-dio
10	5.279	5.225	5.450	348091	0.05	62978	0.07	5.53 CH2=C(CH3)CH2COOH
11	5.508	5.455	5.550	127735	0.02	36219	0.04	3.53 trans-4-hydroxymethyl-2-methy
12	5.577	5.550	5.615	77419	0.01	22266	0.03	3.48 ethanone, 1-(2-furanyl)-
13	5.753	5.615	5.795	3709195	0.50	484161	0.54	7.66 2-hydroxypropanoic acid
14	5.828	5.795	5.885	2404413	0.33	479378	0.54	5.02 .alpha.,.beta.-crotonolactone
15	5.937	5.885	6.100	4892913	0.66	628070	0.71	7.79 1,2-cyclopentanedione
16	6.143	6.100	6.175	898375	0.12	219673	0.25	4.09 2(5h)-furanone, 5-methyl- (identit
17	6.212	6.175	6.250	934422	0.13	217895	0.25	4.29 2,5-furandione, 3-methyl-
18	6.284	6.250	6.345	1116028	0.15	208610	0.23	5.35 1,2-propanediol, 3-chloro-
19	6.417	6.345	6.620	8968547	1.22	1130280	1.27	7.93 2-furancarboxaldehyde, 5-methyl-
20	6.695	6.620	6.840	5130148	0.70	586272	0.66	8.75 2,4-dihydroxy-2,5-dimethyl-3(2h)-furan-3-on
21	6.885	6.840	6.935	1848466	0.25	338342	0.38	5.46 2-propanol, 1,3-dichloro-
22	6.984	6.935	7.030	1824069	0.25	330435	0.37	5.52 phenol
23	7.087	7.030	7.215	3998991	0.54	463891	0.52	8.62 3-methyl hydantoin
24	7.255	7.215	7.385	2256892	0.31	288721	0.32	7.82 glyceraldehyde
25	7.650	7.385	7.685	4185086	0.57	290433	0.33	14.41 glycerin
26	7.763	7.685	7.840	4746370	0.64	826288	0.93	5.74 3-hydroxy-4,4-dimethyldihydro-2(
27	7.880	7.840	7.975	2796058	0.38	427166	0.48	6.55 3-buten-2-one
28	8.026	7.975	8.145	2685742	0.36	290061	0.33	9.26 acetamide, n-(2-methylpropyl)-
29	8.170	8.145	8.225	1065555	0.14	231988	0.26	4.59 2,5-dimethylfuran-3,4(2h,5h)-dione
30	8.258	8.225	8.340	1300284	0.18	209472	0.24	6.21 3-deoxy-d-mannoic lactone
31	8.501	8.340	8.885	21604121	2.93	1675396	1.89	12.89 1-propanone, 1-(2-furanyl)-
32	8.946	8.885	9.140	15194773	2.06	2833887	3.19	5.36 levoglucosenone
33	9.170	9.140	9.335	4514294	0.61	464642	0.52	9.72 aluminum, triethyl-
34	9.515	9.335	9.720	19421933	2.63	2480227	2.79	7.83 2,3-dihydro-3,5-dihydroxy-6-methy
35	9.752	9.720	9.825	3220117	0.44	518730	0.58	6.21 2,5-anhydro-3-o-acetyl-1,4,6-tri-o-methyl-d-
36	9.977	9.825	10.050	8584480	1.16	867000	0.98	9.90 5-[(4-nitrophenoxy)methyl]-2-fur
37	10.156	10.050	10.210	7449448	1.01	837930	0.94	8.89 benzoic acid
38	10.317	10.210	10.430	13048880	1.77	1165146	1.31	11.20 2-amino-9-(3,4-dihydroxy-5-hydrox
39	10.460	10.430	10.515	4783420	0.65	957928	1.08	4.99 piracetam
40	10.604	10.515	10.675	10706826	1.45	1223104	1.38	8.75 1,4:3,6-dianhydro-.alpha.-d-

								glucopyranose
41	10.746	10.675	10.760	8242730	1.12	1924644	2.17	4.28 1,2-benzenediol
42	10.802	10.760	10.870	12280319	1.66	2232224	2.51	5.50 benzofuran, 2,3-dihydro-
43	11.009	10.870	11.425	104864935	14.21	8911817	10.03	11.77 5-(hydroxymethyl)-2-furaldehyde
44	11.492	11.425	11.665	12101634	1.64	1191043	1.34	10.16 cinnamaldehyde, (e)-
45	11.712	11.665	11.850	5916036	0.80	677756	0.76	8.73 phenol, 5-methyl-2-(1-methylethy
46	11.874	11.850	12.005	3494149	0.47	409464	0.46	8.53 isosorbide
47	12.067	12.005	12.120	3769746	0.51	805372	0.91	4.68 2-methoxy-4-vinylphenol
48	12.178	12.120	12.285	8976885	1.22	1224031	1.38	7.33 decanoic acid, 3-methyl-
49	12.299	12.285	12.480	6340491	0.86	736723	0.83	8.61 morpholine, 2,6-dimethyl-
50	12.502	12.480	12.580	1970842	0.27	366271	0.41	5.38 propanoic acid, 2-phenylethyl est
51	12.619	12.580	12.935	4109247	0.56	304125	0.34	13.51 phenol, 2,6-dimethoxy-
52	12.970	12.935	13.025	608514	0.08	115434	0.13	5.27 2-(but-2'-enyl)phenol
53	13.090	13.025	13.120	622727	0.08	110198	0.12	5.65 1-butoxy-1-ethoxyethane
54	13.342	13.120	13.400	3070793	0.42	277527	0.31	11.06 2,8,9-trioxa-5-aza-1-silabicyclo[3.3.3]undeca
55	13.459	13.400	13.590	2882542	0.39	294993	0.33	9.77 vanillin
56	13.797	13.590	13.985	6340706	0.86	389679	0.44	16.27 1,6-anhydro-.beta.-d-talopyranose
57	14.118	13.985	14.210	2058307	0.28	174856	0.20	11.77 1-tetradecanol
58	14.251	14.210	14.290	590570	0.08	126420	0.14	4.67 1,2,3-benzenetriol
59	14.368	14.290	14.445	1187741	0.16	141574	0.16	8.39 2-nonanone
60	14.566	14.445	14.615	1374668	0.19	147265	0.17	9.33 dodecanoic acid, 3-hydroxy-
61	14.666	14.615	14.745	1162803	0.16	173272	0.19	6.71 2(3h)-furanone, 5-(acetyloxy)dihydro-5-meth
62	15.295	14.745	15.480	77105973	10.45	4140747	4.66	18.62 d-allose
63	15.504	15.480	15.610	4860104	0.66	763887	0.86	6.36 4-methyl-2,5-dimethoxybenzalde
64	15.652	15.610	15.710	3043084	0.41	526095	0.59	5.78 2(3h)-furanone, 5-(acetyloxy)dihydro-5-meth
65	15.845	15.710	16.325	32841452	4.45	2440491	2.75	13.46 3-hydroxy-4-methoxybenzoic acid
66	16.505	16.325	17.030	44815037	6.07	1934473	2.18	23.17 1,6-anhydro-.beta.-d-glucofurano
67	17.153	17.030	17.275	8963318	1.21	797764	0.90	11.24 1-isobutyl-7,7-dimethyl-hexahydr
68	17.389	17.275	17.605	10425135	1.41	731822	0.82	14.25 2-furoic acid, tbdms derivative
69	17.689	17.605	17.795	4312285	0.58	710714	0.80	6.07 tetradecanoic acid
70	17.866	17.795	17.925	1648787	0.22	226971	0.26	7.26 hydrocinnamic acid, bornyl ester
71	17.935	17.925	18.040	891549	0.12	176113	0.20	5.06 hexadecane
72	18.061	18.040	18.120	369969	0.05	88388	0.10	4.19 7-thiabicyclo[4.1.0]heptane
73	18.169	18.120	18.225	533257	0.07	128071	0.14	4.16 heptasiloxane, hexadecamethyl-
74	18.265	18.225	18.280	149491	0.02	47340	0.05	3.16 2,6-octadiene-1,8-diol, 2,6-dimethyl-
75	18.330	18.280	18.400	413189	0.06	75241	0.08	5.49 4-hydroxy-3,5,6-trimethyl-4-[(1e)-3-
76	18.514	18.400	18.585	654284	0.09	74726	0.08	8.76 pyrazino[2,3-c][1,2,5]-oxadiazole, 5,6-bis(iso
77	18.706	18.585	18.735	550281	0.07	69623	0.08	7.90 1-methoxy-3-hydroxymethylheptane
78	18.793	18.735	18.905	1184249	0.16	216825	0.24	5.46 tetradecanoic acid
79	18.955	18.905	18.990	442309	0.06	114412	0.13	3.87 1-tetradecanol
80	19.020	18.990	19.040	249363	0.03	89714	0.10	2.78 n-pentadecanol
81	19.082	19.040	19.155	580988	0.08	137179	0.15	4.24 heneicosane
82	19.207	19.155	19.250	247913	0.03	57214	0.06	4.33 2-butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclo
83	19.274	19.250	19.365	153133	0.02	33730	0.04	4.54 2-ethylbutyric acid, 3-methylpent-2-yl ester
84	19.411	19.365	19.470	130349	0.02	44647	0.05	2.92 pentadecanoic acid, methyl ester
85	19.580	19.470	19.635	752321	0.10	173016	0.19	4.35 5-(3-hydroxypropyl)-2,3-dimethoxyphenol
86	19.683	19.635	19.765	674490	0.09	124798	0.14	5.40 2-nonadecanone
87	19.876	19.765	20.040	22767593	3.08	5398571	6.07	4.22 n-hexadecanoic acid

88	20.105	20.040	20.225	2285280	0.31	332079	0.37	6.88 hexadecanoic acid, ethyl ester
89	20.294	20.225	20.310	532538	0.07	126347	0.14	4.21 1,6-heptadiene, 2-methyl-6-phenyl-
90	20.325	20.310	20.420	488268	0.07	124989	0.14	3.91 4-({2-[2-ethyl-2-methyl-4-(4-methyl
91	20.477	20.420	20.515	749795	0.10	223481	0.25	3.36 naphthalene, 2,6-bis(1,1-dimethylethyl)-
92	20.557	20.515	20.585	1085516	0.15	349435	0.39	3.11 1,6-heptadiene, 2-methyl-6-phenyl-
93	20.626	20.585	20.705	1928901	0.26	499509	0.56	3.86 1-phenylbicyclo(4.1.0)heptane
94	20.738	20.705	20.805	409596	0.06	82087	0.09	4.99 1,6-heptadiene, 2-methyl-6-phenyl-
95	20.857	20.805	20.900	589328	0.08	156224	0.18	3.77 1-(4-ethoxyphenyl)-3-phenyl-bute
96	20.951	20.900	21.005	670699	0.09	154701	0.17	4.34 1-hexacosene
97	21.061	21.005	21.100	666879	0.09	161439	0.18	4.13 n-nonadecanol-1
98	21.128	21.100	21.235	742913	0.10	164797	0.19	4.51 heneicosane
99	21.301	21.235	21.380	936175	0.13	247035	0.28	3.79 13-docosenoic acid, methyl ester
100	21.441	21.385	21.470	75479	0.01	21856	0.02	3.45 octadecanoic acid, methyl ester
101	21.528	21.470	21.555	201383	0.03	56133	0.06	3.59 2,2-dideutero octadecanal
102	21.662	21.555	21.680	14590145	1.98	3732598	4.20	3.91 9,12-octadecadienoic acid (z,z)-
103	21.750	21.680	21.825	31107754	4.21	4912860	5.53	6.33 octadec-9-enoic acid
104	21.863	21.825	21.995	15446051	2.09	2682482	3.02	5.76 octadecanoic acid
105	22.064	21.995	22.145	6693361	0.91	820811	0.92	8.15 benzene, 1,4-dihexadecyl-
106	22.160	22.145	22.300	4523733	0.61	592889	0.67	7.63 4,5-diphenylocta-1,7-diene(dl)
107	22.339	22.300	22.385	1967333	0.27	401304	0.45	4.90 hydrazine, (2-phenylethyl)-
108	22.423	22.385	22.510	2576030	0.35	377401	0.42	6.83 9,12-octadecadienoic acid (z,z)-
109	22.545	22.510	22.590	1438861	0.19	329938	0.37	4.36 hexadecanoic acid, 2-hydroxyethyl ester
110	22.635	22.590	22.700	2139925	0.29	427312	0.48	5.01 1h-purin-6-amine, [(2-fluoropheny
111	22.720	22.700	22.780	1076848	0.15	246627	0.28	4.37 n,n-dimethyloctadecanamide
112	22.821	22.780	22.870	1060153	0.14	211889	0.24	5.00 1-octadecene
113	22.966	22.870	23.025	2875066	0.39	426618	0.48	6.74 undecanal, 2-methyl-
114	23.077	23.025	23.180	2893365	0.39	434982	0.49	6.65 5-(1-phenyl-cyclopentyl)-[1,3,4]ox
115	23.204	23.180	23.235	731683	0.10	224838	0.25	3.25 e,e,z-1,3,12-nonadecatriene-5,14-diol
116	23.276	23.235	23.295	819350	0.11	233517	0.26	3.51 oxazole, 2,2'-(1,4-butanediyl)bis[4,5
117	23.374	23.295	23.445	2311495	0.31	298048	0.34	7.76 (17.alpha.)-19-norpregh-4,7-dien-20-
118	23.503	23.445	23.595	2119220	0.29	256454	0.29	8.26 7-heptadecene, 17-chloro-
119	23.748	23.595	23.795	3280924	0.44	377636	0.42	8.69 2,5-di(trifluoromethyl)benzoic acid, 3-hexade
120	23.858	23.795	23.925	2406754	0.33	432732	0.49	5.56 1h-purin-6-amine, [(2-fluoropheny
121	23.979	23.925	24.020	1210249	0.16	217311	0.24	5.57 oleic anhydride
122	24.208	24.020	24.295	5362421	0.73	535669	0.60	10.01 oleoyl chloride
123	24.340	24.295	24.415	2215355	0.30	338967	0.38	6.54 eicosanoic acid, 2-hydroxyethyl
124	24.466	24.415	24.535	2282042	0.31	372859	0.42	6.12 fumaric acid, 2-dimethylaminoethyl nonyl este
125	24.598	24.535	24.625	1509711	0.20	284972	0.32	5.30 2-[2-carboxyethyl]-3-methyl-tetrahydrofurano
126	24.725	24.625	24.840	4795429	0.65	563759	0.63	8.51 hexadecanoic acid, 2-hydroxy-1-(
127	24.892	24.840	24.945	1814030	0.25	315824	0.36	5.74 triethylene glycol monododecyl ether
128	25.033	24.945	25.095	2662664	0.36	365088	0.41	7.29 cyclononasiloxane, octadecamethyl-
129	25.110	25.095	25.255	1990625	0.27	233665	0.26	8.52 glutaric acid, di((5-ethyl-1,3-dioxan-5-yl)meth
130	25.306	25.255	25.355	1168853	0.16	208462	0.23	5.61 bis(2-ethylhexyl) phthalate
131	25.411	25.355	25.490	1449908	0.20	189417	0.21	7.65 1-heptatriacotanol
132	25.589	25.490	25.700	2255655	0.31	239546	0.27	9.42 triacontane, 1-iodo-
133	25.781	25.700	25.815	942976	0.13	144123	0.16	6.54 1,2-dichloro-2-phenyl-1-(4-chloroethynyl)phe
134	25.880	25.815	25.935	957170	0.13	141265	0.16	6.78 benzene, nonadecyl-

135	26.025	25.935	26.070	1023737	0.14	135487	0.15	7.56 hexadecadienoic acid, methyl es
136	26.132	26.070	26.230	1441749	0.20	235291	0.26	6.13 1,3-dioxolane, 4-[[(2-methoxyhexa
137	26.250	26.230	26.280	279415	0.04	93998	0.11	2.97 methyl 3-[(trifluoroacetoxy)methylthio]prop-2
138	26.336	26.280	26.385	823628	0.11	192806	0.22	4.27 cyclononasiloxane, octadecamethyl-
139	26.470	26.385	26.540	1599894	0.22	264446	0.30	6.05 9,12-octadecadienoic acid (z,z)-, 2-hydroxy-
140	26.569	26.540	26.635	590012	0.08	135960	0.15	4.34 hexadecane, 2-methyl-
141	26.761	26.635	26.840	1341549	0.18	190676	0.21	7.04 9-octadecenoic acid, 1,2,3-propanetriyl ester,
142	26.887	26.840	26.975	409873	0.06	77660	0.09	5.28 diethylene glycol monolaurate
				738123183	100.00	88875070	100.00	

CONCLUSION

The present study unequivocally establishes the antioxidant activity of the Zizania aquatica extract, indicating potential health benefits associated with its diverse phytoconstituents. While these finding are promising, further research is warranted to comprehensively explore and understand the extract varied pharmacological activity.

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