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Comparative evaluation of phytoconstituents and antioxidant potential in *Aloe barbadensis*, *Cereus hexagonus*, and *Cissus quadrangularis* ethanol extracts**D. Ruffina*, L. Prince**

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ABSTRACT

Plant-derived secondary metabolites are increasingly explored for their antioxidant potential, prompting this study to evaluate the phytochemical composition and antioxidant activity of ethanol extracts from *Aloe barbadensis*, *Cereus hexagonus*, and *Cissus quadrangularis*. Qualitative phytochemical screening revealed carbohydrates, flavonoids, glycosides, and phenols in all three extracts, with alkaloids absent; *Cissus quadrangularis* exhibited the richest phytochemical profile, followed by *A. barbadensis* and *C. hexagonus*. GC-MS profiling identified 78, 25, and 86 peaks respectively, with major compounds including fatty acids (palmitic, oleic, linoleic), sterols (campesterol, stigmasterol, γ -sitosterol), triterpenoids (α -amyrin, β -amyrin, lupenyl derivatives), and phenolics. Antioxidant assays demonstrated concentration-dependent activity: in DPPH radical scavenging, *C. quadrangularis* showed the highest activity at 500 μ g/mL (88.5% RSA), while *C. hexagonus* exhibited superior activity at lower concentrations (62.8–72.9% RSA at 100–300 μ g/mL); in reducing power assay, *A. barbadensis* demonstrated the strongest activity (85% RSA at 500 μ g/mL), comparable to ascorbic acid (83.5%). These findings indicate that all three plant extracts possess significant antioxidant activity attributable to their diverse phytochemical constituents, particularly phenolics, flavonoids, fatty acids, and triterpenoids, supporting their potential applications in nutraceutical and pharmaceutical formulations.

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INTRODUCTION

Since ancient times, plants have been utilized as sources of medicine due to the bioactive phytochemicals they harbor (Sinha *et al.*, 2022). These naturally occurring substances, present in various parts of the plant such as roots, bark, leaves, and seeds, are chiefly responsible for the therapeutic effects attributed to plants. The processes of extracting, quantifying, and analyzing phytochemicals are critical for the identification of new drug candidates and for ensuring the quality of products derived from medicinal plants (Ingle *et al.*, 2017). Phytochemicals are essential in protecting plants against pathogens and environmental stressors, while also affecting their distinctive color, aroma, and flavor (Haseen *et al.*, 2024). Recent advancements in phytochemical analysis techniques, including extraction, chromatography, and spectroscopy, allow researchers to assess medicinal properties, discover new compounds, and enhance quality control measures. A comprehensive understanding of plant chemistry is vital for disease prevention, boosting immunity, and the development of innovative therapeutics.

Aloe vera (syn. *Aloe barbadensis* Miller) belongs to the Xanthorrhoeaceae family and is a perennial succulent plant (Nyffeler, 2001). Out of more than 400 species, only a few species of *Aloe* have been considered for commercial importance. *A. vera* being the most potent and, thereby, the most popular plant in the research as well as marketing field (Eshun and He, 2004). *A. vera* is a rich source of over 200 naturally occurring nutrients which are water soluble and fat soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids (Boudreau and Beland, 2006). Its secondary metabolites have multiple properties such as anti-inflammatory, antibacterial, antioxidant, immune boosting, anticancer, anti-ageing, sunburn relief and antidiabetic potentials (Rishi *et al.*, 2008; Wintola and Afolayan, 2011; Langmead *et al.*, 2004). Several traditional uses also have been reported such as for burn injury, eczema, cosmetics, inflammation, fever etc. (Jayaprakasha *et al.*, 2001).

Cereus hexagonus, commonly known as the lady-of-the-night cactus, is traditionally utilized in herbal and folk medicine where extracts from its stems and flowers are valued for their potential cardiac-stimulating, diuretic, and anti-inflammatory properties. Cardiac Support: Folk remedies use parts of the plant as a natural heart tonic or stimulant to address weak heart function. Urinary and Diuretic Uses: Traditional practices employ the plant to help manage bladder infections and promote fluid elimination. Pain Relief: Smashed or prepared stem tissues are occasionally applied topically to ease joint discomfort.

Cissus quadrangularis (CQ) is a succulent plant of the family "Vitaceae". It has been cited in ancient Ayurveda texts by Bhava Prakasha and Chakra Dutta as a general tonic, especially for fractured bones. Additionally to its anti-osteoporotic activity, CQ exhibits many medicinal activities such as anti-arthritic, anti-diabetic, gastroprotective, antioxidant, anti-obesity, analgesic, anti-inflammatory and antipyretic activity (Bhujade *et al.*, 2015; Lee *et al.*, 2018). Versatile constituents are responsible for these activities. Phytochemical studies of CQ have shown the presence of various versatile constituents such as flavonoids, triterpenoids, saponins, phytosterols, stilbene derivatives etc. (Kaur *et al.*, 2021).

This present study focuses on comparative phytochemical analysis, total phenolic content and antioxidant potential of methanolic extracts of *A. vera*, *Cereus hexagonus* and *Cissus quadrangularis*. These vast variations may cause a difference in the phytoconstituents of the plant species. This study is the first attempt to evaluate antioxidant potential of different *A. vera* accessions from different plant of India.

MATERIALS AND METHODS

Collection and preparation of plant materials

Healthy cladodes of *Aloe barbadensis*, *Cereus hexagonus* and *Cissus quadrangularis* were collected from Kolunji Ecological farm, Keeranur during February 2023.

The collected samples were washed thoroughly with tap water followed by distilled water to remove adhering soil and debris. After cleaning, the materials were cut into small pieces and shade-dried at room temperature until a constant weight was achieved. The dried samples were ground to a fine powder using a laboratory grinder and stored in airtight containers until extraction.

Preparation of ethanol extracts

Approximately 20 g of each powdered plant material was subjected to Soxhlet extraction with 250 mL of ethanol for 24 h. The resulting extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40–45°C. The concentrated extracts were further dried and stored at 4°C in sterile, labelled containers until use (Yadav and Agarwala, 2011).

For phytochemical and antioxidant assays, the dried extracts were dissolved in ethanol and diluted to the required concentrations. The extraction yield was calculated using the standard formula.

Qualitative phytochemical screening

The ethanol extracts were screened for the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, steroids, tannins, and terpenoids using standard qualitative procedures (Harborne and Williams, 1967).

Preliminary screening included Dragendorff's or Mayer's test for alkaloids, ferric chloride test for phenols and tannins, foam test for saponins, Shinoda or alkaline reagent test for flavonoids, Borntrager's test for anthraquinones, and Liebermann–Burchard or Salkowski tests for steroids and terpenoids. Results were recorded as positive (+) when a characteristic colour change or precipitate was observed and negative (–) in the absence of a visible reaction.

Alkaloid test

About 1 mL of extract was acidified with dilute hydrochloric acid and filtered. The filtrate was treated separately with Mayer's reagent and Dragendorff's

reagent. Cream or reddish-brown precipitate indicated alkaloids.

Anthraquinone test

The extract was treated with dilute sulphuric acid and gently heated. After cooling, the mixture was filtered and extracted with an organic solvent. The organic layer was then treated with dilute ammonia solution. A pink, red, or violet colour in the alkaline layer indicated anthraquinones.

Carbohydrate test

The extract was treated with Molisch's reagent, followed by careful addition of concentrated sulphuric acid along the side of the test tube. A violet ring at the junction of the two layers indicated carbohydrates.

Flavonoid test

The extract was mixed with a few drops of sodium hydroxide solution. An intense yellow colour that disappeared upon addition of dilute hydrochloric acid was taken as a positive result for flavonoids.

Glycoside test

The extract was treated with glacial acetic acid, ferric chloride solution, and concentrated sulphuric acid. Formation of a brown ring at the interface or a characteristic colour change suggested the presence of glycosides.

Phenol test

A small quantity of extract was treated with a few drops of ferric chloride solution. Development of a blue, green, or violet colour indicated phenolic compounds.

Protein test

The extract was treated with sodium hydroxide solution followed by a few drops of copper sulphate solution. A violet or purple colour indicated proteins.

Saponin test

The extract was mixed with distilled water and vigorously shaken in a graduated test tube. Persistent froth that remained for several minutes indicated saponins.

Steroid test

The extract was dissolved in chloroform and carefully treated with acetic anhydride followed by concentrated sulphuric acid. A green or bluish-green colour indicated steroids.

Tannin test

The extract was treated with ferric chloride solution. A blue, green, or bluish-black colour indicated tannins.

Terpenoid test

The extract was mixed with chloroform, followed by careful addition of concentrated sulphuric acid. A reddish-brown colour at the interface indicated terpenoids.

DPPH radical-scavenging assay

The free-radical-scavenging activity of the ethanol extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Choi *et al.*, 2002). DPPH, a stable purple free radical, is reduced in the presence of antioxidants, resulting in a decrease in absorbance typically measured at 517 nm.

A fresh DPPH solution (0.1 mM in ethanol) was prepared and protected from light. Plant extracts were prepared at concentrations of 100, 200, 300, 400, and 500 µg/mL. One millilitre of each extract concentration was mixed with 1 mL of DPPH solution, vortexed, and incubated in the dark at room temperature for 20–30 min.

Absorbance was measured at 517 nm using a UV–visible spectrophotometer under standard laboratory conditions. Ascorbic acid served as the standard antioxidant for comparison. A reagent control containing DPPH solution without plant extract was also prepared. All assays were performed in triplicate, and the percentage of radical-scavenging activity was calculated using the standard formula, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract or standard. Higher percentage inhibition indicated greater DPPH radical-scavenging activity of the tested samples.

Reducing-power assay

The reducing power of the ethanol extracts was assessed based on their ability to reduce ferric ions to ferrous ions (Wong *et al.*, 2006). Extract concentrations ranging from 100 to 500 µg/mL were prepared in ethanol.

One millilitre of each extract concentration was mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide. The reaction mixture was incubated at 50°C for 20 min. After incubation, 1 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 min.

The upper layer was mixed with distilled water and 0.1% ferric chloride solution. After 10 min of incubation, absorbance was measured at 700 nm. Increased absorbance indicated greater reducing power. Ascorbic acid was used as the reference standard.

Data analysis

All experiments were conducted in triplicate, and results were expressed as mean $\pm$ standard deviation. Antioxidant activity was plotted against extract concentration, and the activity of plant extracts was compared with that of ascorbic acid. Where appropriate, statistical analysis was performed using one-way analysis of variance (ANOVA) followed by a suitable post-hoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Qualitative phytochemical screening

Qualitative phytochemical analysis revealed the presence of carbohydrates, flavonoids, glycosides, and phenols in the ethanol extracts of all three plants (Table 1). Alkaloids were absent in all the extracts. The *Aloe barbadensis* extract contained anthraquinones, carbohydrates, flavonoids, glycosides, phenols, steroids, and terpenoids. The *Cereus hexagonus* extract showed carbohydrates, flavonoids, glycosides, phenols, proteins, and tannins. The *Cissus quadrangularis* extract contained carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, steroids and terpenoids.

Table 1. Qualitative phytochemical constituents of the ethanol extracts of *Aloe barbadensis*, *Cereus hexagonus* and *Cissus quadrangularis*

Phytocompounds	<i>Aloe barbadensis</i>	<i>Cereus hexagonus</i>	<i>Cissus quadrangularis</i>
Alkaloid	-	-	-
Anthraquinone	+	-	-
Carbohydrate	+	+	+
Flavonoid	+	+	+
Glycosides	+	+	+
Phenol	+	+	+
Protein	-	+	+
Saponin	-	-	+
Steroid	+	-	+
Tannin	-	+	-
Terpenoids	+	-	+

Among the three plants, *Cissus quadrangularis* showed the highest number of phytochemical constituents, followed by *Aloe barbadensis* and *Cereus hexagonus*. Anthraquinones, tannins, and saponins were detected exclusively in *Aloe vera*, *Cereus hexagonus*, and *Cissus quadrangularis*, respectively. The convergence of qualitative and GC-MS data reinforces the richness of secondary metabolites, particularly phenolics, flavonoids and terpenoid compounds, which are known contributors to antioxidant activity.

GC-MS phytochemical profiling

Gas chromatography–mass spectrometry (GC-MS) analysis of the ethanol extracts of *Aloe barbadensis*, *Cereus hexagonus*, and *Cissus quadrangularis* revealed a diverse array of phytoconstituents, with notable differences in chemical class distribution among the three species (Fig. 1-3, Table 2-4).

Aloe barbadensis

The ethanol extract of *A. barbadensis* yielded 78 detectable peaks, with major compounds belonging to carbohydrates, organic acids, phenolics, fatty acids, and steroidal/triterpenoid derivatives. The most abundant constituents included 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (95.10% area), 9,12-octadecadienoyl chloride (Z,Z)- (99.68%), 1-heptatriacotanol (100.00%), n-hexadecanoic acid (78.44%), and 9,12-octadecadienoic acid (Z,Z)- (66.08%). Other significant compounds were glycerin (17.43%), thymine (11.13%), 5-hydroxymethylfurfural (11.35%), lidocaine (22.86%), phytol (12.61%), γ -tocopherol (15.27%), vitamin E (5.50%), campesterol (22.15%), and stigmasterol (17.63%). These findings align with previous metabolomic studies reporting sugars, organic acids, anthraquinone derivatives, fatty acids, and sterols as characteristic metabolites of *Aloe barbadensis* gel and leaf extracts.

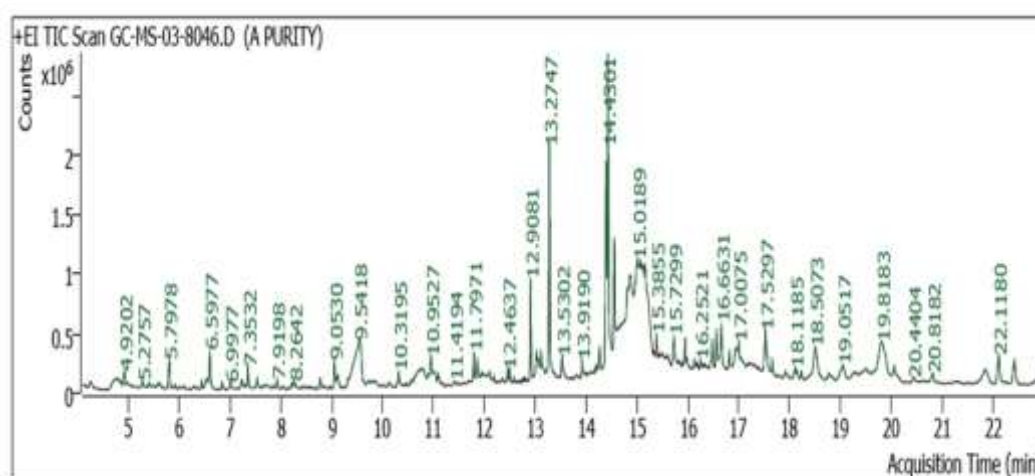
**Fig. 1.** GC-MS analysis of the ethanol extracts of *Aloe barbadensis*

Table 2. GC-MS analysis of the ethanol extracts of *Aloe barbadensis*

Peak	RT	Area%	Compound name
1	4.2536	3.22	1,2-Cyclopentanedione
2	4.7757	17.43	Glycerin
3	4.9202	12.67	Formic acid, 2-propenyl ester
4	5.2757	3.27	Benzyl chloride
5	5.3979	4.37	Piperidine, 1-nitroso-
6	5.6090	3.76	Acetamide, N-[4-(chlorodifluoromethoxy)phenyl]-2-morpholin-4-yl-
7	5.7978	11.13	Thymine
8	5.9089	0.94	1,3-Dioxane, 2,4-dimethyl-
9	6.0867	2.40	Diazene, bis(1,1-dimethylethyl)-
10	6.4422	2.28	Ethanamine, N-ethyl-N-nitroso-
11	6.5977	21.84	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
12	6.8422	2.38	(S)-5-Hydroxymethyl-2[5H]-furanone
13	6.9977	4.40	Pentanoic acid, 2-isopropoxyphenyl ester
14	7.2310	6.87	2(3H)-Furanone, 5-hexyldihydro-
15	7.3532	8.87	5-Hydroxymethylfurfural
16	7.5310	5.52	1,2,3-Propanetriol, 1-acetate
17	7.7309	4.79	1,2,3,4-Butanetetrol, [S-(R*,R*)]-
18	7.8420	1.28	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
19	7.9198	2.52	1-(Methylthio)-3-pentanone
20	8.0309	1.08	2-t-Butyl-4-methyl-5-oxo-[1,3]dioxolane-4-carboxylic acid
21	8.2642	5.50	2-Methoxy-4-vinylphenol
22	8.7752	3.06	1,2,4-Benzenetriol
23	9.0530	8.37	3-[N'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine
24	9.1196	6.93	Butanamide, 2-hydroxy-N,2,3,3-tetramethyl-
25	9.5418	95.10	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
26	9.7973	10.43	Spiro[bicyclo[3.2.1]oct-3-ene-2,2'-[1,3]dioxolane]
27	10.1195	1.55	Tetraacetyl-d-xylonic nitrile
28	10.3195	5.25	Dodecanoic acid
29	10.7972	37.66	3-Deoxy-d-mannonic lactone
30	10.9527	26.95	Ethyl .alpha.-d-glucopyranoside
31	11.0972	3.77	Phenol, 4-(ethoxymethyl)-2-methoxy-
32	11.4194	1.88	d-Mannose
33	11.7971	9.20	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
34	11.8637	10.27	Tetradecanoic acid
35	11.9415	11.35	Adenosine-3-phosphoric acid
36	12.0970	7.76	2-Methylcyclohexan-1-on-3-ylbutanoic acid, 2,2-dimethyl-3 (methylsulfonyloxy) propyl ester
37			Benzoethiophene-2-carboxylic acid, 4,5,6,7-tetrahydro-
38	12.3526	1.69	7-hydroximino-3-[2-(4-morpholyl)-1-oxoethylamino]-, ethyl ester
39	12.4637	6.03	Phytol
40	12.9081	22.86	Lidocaine
41	13.0303	12.31	1H-[1]Pyridine-3-carbonitrile, 4-ethyl-2-oxo-2,5,6,7-tetrahydro-
42	13.1191	4.05	2,5-Octadecadienoic acid, methyl ester
43	13.2747	78.44	n-Hexadecanoic acid
44	13.5302	5.94	1-Propyl-3,6-diazahomoadamantan-9-ol
45	13.9190	5.39	Heptadecanoic acid
46	14.1968	7.34	1-Chloroeicosane
47	14.2634	12.61	Phytol
48	14.3968	66.08	9,12-Octadecadienoic acid (Z,Z)-
49	14.4301	99.68	9,12-Octadecadienoyl chloride, (Z,Z)-
50	14.5523	25.85	Octadecanoic acid
51	14.8634	47.59	1-Heptatriacotanol
52	15.0189	100.00	1-Heptatriacotanol
53	15.3855	3.79	Heptacosane
54	15.7299	10.22	Eicosanoic acid
55	15.9521	6.75	Heptacosane
56	16.1299	3.67	Octadecane, 1-(ethenyl)-
57	16.2521	2.40	Benzenepropanol, .alpha.-methyl-.beta.-nitro-, (R*,R*)-(+,-)-
58	16.4854	7.39	Heptacosane
59	16.5631	10.92	Glycerol 1-palmitate
60	16.6631	19.25	Benzyl-diethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate
61	16.8187	5.19	Docosanoic acid
62	17.0075	36.68	Heptacosane, 1-chloro-
63	17.5297	23.58	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate
64	17.6630	4.17	Octadecanoic acid, 2,3-dihydroxypropyl ester
65	17.9185	3.36	Tetracosanoic acid

66	18.1185	7.27	Heptacosane, 1-chloro-
67	18.2407	2.00	Supraene
68	18.5073	36.23	Bi-1-cyclohexen-1-yl, 3,3,3',3',5,5,5',5'-octamethyl-
69	18.7740	4.25	Ethanol, 2-(octadecyloxy)-
70	19.0517	16.89	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
71	19.5183	36.19	9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-
72	19.8183	88.68	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
73	20.0627	15.27	gamma.-Tocopherol
74	20.4404	2.23	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-
75	20.8182	5.50	Vitamin E
76	21.8514	22.99	9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-
77	22.1180	22.15	Campesterol
78	22.4291	17.63	Stigmasterol

Cereus hexagonus

GC-MS analysis of *C. hexagonus* ethanol extract identified 25 peaks, dominated by fatty acids and their trimethylsilyl (TMS) derivatives. The major constituents were oelsaeure (oleic acid) trimethylsilylester (20.80% and 3.32%), 9,12-octadecadienoic acid (Z,Z)- TMS derivative (12.83%), hexadecanoic acid (palmitic acid) trimethylsilyl derivative (7.78%), linoleyl acetate (5.52%), 9-

octadecenoic acid (Z)- oxiranylmethyl ester (7.22%), tetrapentacontane (6.63%), behenic acid TMS derivative (1.70%), and arachidic acid TMS derivative (1.91%). The prevalence of long-chain saturated and unsaturated fatty acids (palmitic, oleic, linoleic, stearic, arachidic, behenic) is consistent with the lipid-rich nature of cactus species, where fatty acids contribute to membrane integrity and stress tolerance.

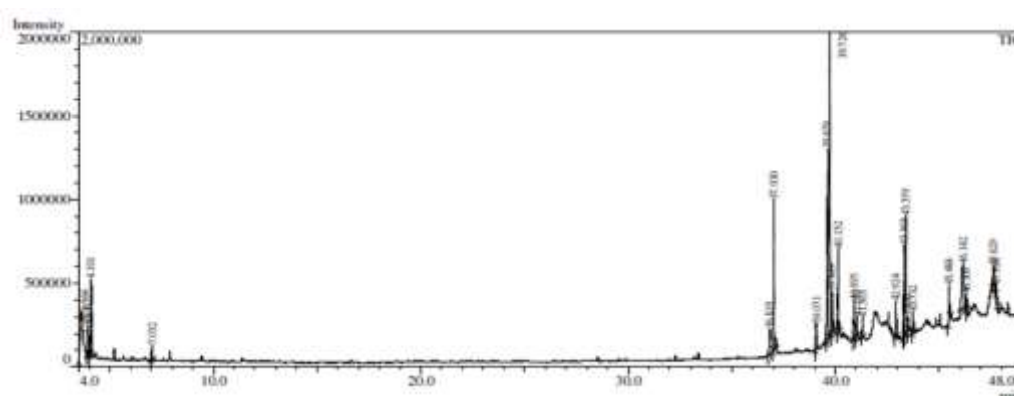


Fig. 2. GC-MS analysis of the ethanol extracts of *Cereus hexagonus*

Table 3. GC-MS analysis of the ethanol extracts of *Cereus hexagonus*

Peak	RT	Area%	Compound name
1	3.566	3.28	Trifluoroacetamide, TMS derivative
2	3.937	1.14	cis-3-Hexenyllactate
3	4.010	0.81	N,N-Diethyl(trimethylsilyl)carbamate
4	4.101	5.26	cis-3-Hexenyllactate
5	7.032	0.83	SILANOL, TRIMETHYL-, TRIESTER WIT
6	36.835	3.78	Palmitic Acid, TMS derivative
7	37.030	7.78	HEXADECANOIC ACID, TRIMETHYLSI
8	39.071	1.21	Phytol, TMS derivative
9	39.629	12.83	9,12-OCTADECADIENOIC ACID (Z,Z)-, T
10	39.726	20.80	OELSAEURE, TRIMETHYLSIYLESTER
11	39.829	3.32	OELSAEURE, TRIMETHYLSIYLESTER
12	40.132	4.90	Stearic acid, TMS derivative
13	40.895	2.78	Glycidyl palmitate
14	41.017	1.42	LINOLSAEURE, TRIMETHYLSIYLEST
15	41.303	1.00	10-Nonadecenoic acid, (Z)-, TMS derivative
16	42.924	1.91	Arachidic acid, TMS derivative
17	43.303	5.52	Linoleyl acetate
18	43.379	7.22	9-Octadecenoic acid (Z)-, oxiranylmethyl est

19	43.490	0.72	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-
20	43.732	1.22	Myristic acid glycidyl ester
21	45.488	1.70	Behenic acid, TMS derivative
22	46.142	6.63	Tetrapentacontane
23	46.309	0.71	HEXATRIACONTANE
24	47.629	2.45	2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-1,1
25	47.764	0.78	HEXATRIACONTANE
		100.00	

Cissus quadrangularis

The ethanol extract of *C. quadrangularis* showed 86 peaks, with a chemically diverse profile including carbohydrates, organic acids, phenolics, fatty acids, triterpenoids, and sterols. Dominant compounds were 9,12-octadecadienoyl chloride (Z,Z)- (100.00%), lup-20(29)-en-3-ol acetate (3 β) (92.85%), α -amyrin (60.77% and 9.86%), n-hexadecanoic acid (64.48%), γ -sitosterol (55.28%), 1-heptatriacotanol (76.66%), 3-deoxy-D-mannonic

lactone (33.59%), glycerin (35.28%), and 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (78.65%). Notably, triterpenoids such as α -amyrin, β -amyrin (16.95%), and lupenyl acetate derivatives were prominent, alongside sterols (γ -sitosterol, campesterol, stigmasterol) and long-chain alkanes. These results corroborate earlier reports identifying triterpenoids, flavonoids, steroids, and phenolics as key bioactive constituents of *C. quadrangularis* ethanolic extracts.

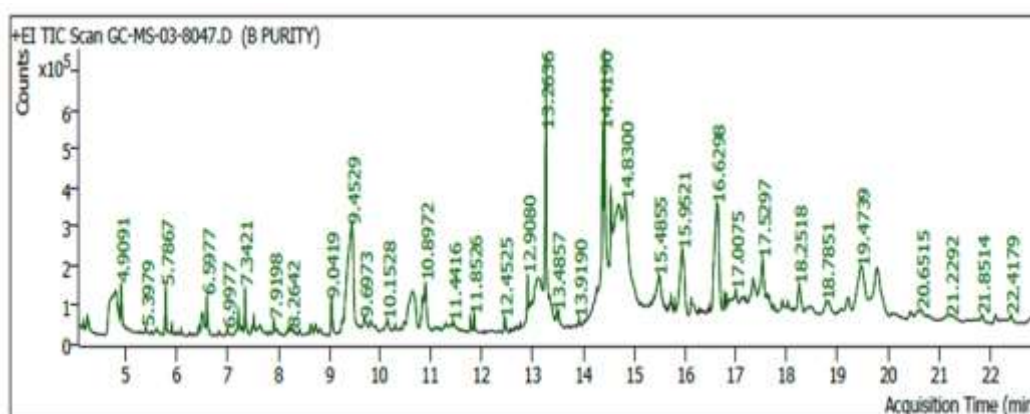


Fig. 3. GC-MS analysis of the ethanol extracts of *Cissus quadrangularis*

Table 4. GC-MS analysis of the ethanol extracts of *Cissus quadrangularis*

Peak	RT	Area%	Compound name
1	4.1647	2.68	Pyrrolidine
2	4.2425	6.58	1,2-Cyclopentanedione
3	4.7980	35.28	Glycerin
4	4.9091	10.77	Formic acid, 2-propenyl ester
5			Pentanamide, 2-(dimethylamino)-4-methyl-N-[2-methyl-1-[[3,3a,11,12,13,14,15,15a-octahydro-12,15-b][1,5,8]oxadiazacyclotetradecin-1(2H)-yl]carbonyl]butyl]-
6			(+)-trans-3,4-Dimethyl-2-phenyltetrahydro-1,4-thiazine
7	5.3979	0.66	Thymine
8	5.6090	2.00	1,3-Dioxolane, 4-ethyl-2-pentadecyl-
9	5.7867	7.10	Diazene, bis(1,1-dimethylethyl)-
10	5.9089	1.32	Ethanamine, N-ethyl-N-nitroso-
11	6.0867	0.80	dl-Glyceraldehyde dimer
12	6.4422	1.13	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
13	6.5089	7.48	2(5H)-Furanone, 5-(1-methylethyl)-
14	6.5977	6.56	Catechol
15	6.8310	0.44	2(3H)-Furanone, 5-heptyldihydro-
16	6.9977	0.90	5-Hydroxymethylfurfural
17	7.2199	6.93	Diethoxymethyl acetate
18	7.3421	7.47	
19	7.5198	4.25	

20	7.6420	4.92	Erythritol
21	7.8198	0.41	Muramic acid
22	7.9198	3.66	1-(Methylthio)-3-pentanone
23	8.1975	1.49	Benzenemethanol, .alpha.-[(methylamino)methyl]-
24	8.2642	2.18	2-Methoxy-4-vinylphenol
25	8.4197	0.63	Butanedioic acid, 2-hydroxy-2-methyl-, (S)-
26	8.6419	1.81	Phosphonofluoridic acid, ethyl-, nonyl ester
27	8.7308	2.39	1,2,3-Benzenetriol
28	8.8308	1.11	2-Nitro-4-nonanol
29	9.0419	6.54	3-[N'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine
30	9.4529	78.65	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
31	9.6973	2.42	Dimethyl{bis[(2Z)-pent-2-en-1-yloxy]}silane
32	9.8195	1.57	.beta.-D-Glucopyranose, 1,6-anhydro-
33	10.1528	3.09	Ribitol
34	10.3084	0.67	Dodecanoic acid
35	10.6417	33.59	3-Deoxy-d-mannonic lactone
36	10.8972	22.23	d-Glycero-d-ido-heptose
37	11.0972	1.85	Phenol, 4-(ethoxymethyl)-2-methoxy-
38	11.2971	2.75	d-Mannose
39	11.4416	5.75	17-Octadecynoic acid
40	11.7860	1.83	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
41	11.8526	2.26	Tetradecanoic acid
42	12.4525	1.93	Phytol
43	12.7636	1.29	Ethyl iso-allocholate
44	12.9080	10.15	Lidocaine
45	13.1080	55.28	.gamma.-Sitosterol
46	13.2636	64.48	n-Hexadecanoic acid
47	13.4857	6.16	L-Lyxose
48	13.9190	0.73	Heptadecanoic acid
49	14.4190	100.00	9,12-Octadecadienoyl chloride, (Z,Z)-
50	14.5523	25.73	Octadecanoic acid
51	14.6856	92.85	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
52			1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-
53			tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-
54			(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-
55			triacetate, [1aR-
56	14.8300	76.66	(1a.alpha.,1b.beta.,4a.beta.,5.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-
57	15.4855	16.95	.beta.-Amyrin
58	15.7299	1.08	Eicosanoic acid
59	15.7966	1.42	9,10-Anthracenedione, 1,8-dihydroxy-3-methyl-
60			4,4,6a,6b,8a,11,11,14b-Octamethyl-
61	15.9521	28.44	1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-
			octadecahydro-2H-picen-3-one
62	16.1299	4.38	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
63	16.6298	60.77	.alpha.-Amyrin
64	16.7742	2.58	10-Hydroxy-5-methoxy-2-methyl-1,4-anthracenedione
65	17.0075	18.09	Ethyl iso-allocholate
66			4,4,6a,6b,8a,11,11,14b-Octamethyl-
67	17.3408	19.87	1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-
68	17.5297	31.90	octadecahydro-2H-picen-3-one
69	17.9185	2.93	1-Heptatriacotanol
70			Tetracosanoic acid
71			5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-
72			tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-
73			1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-
			dihydroxy-1,1,6,8-tetramethyl-, [1aR-
			(1a.alpha.,1b.beta.,2.alpha.,3.beta.,4a.beta.,7a.alpha.
74	18.0296	2.39	,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-
75	18.2518	9.86	.alpha.-Amyrin
76	18.4851	3.46	7,8-Epoxyxanostan-11-ol, 3-acetoxy-
77	18.7851	6.14	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-
78	19.2072	7.85	Friedelan-3-one
79	19.4739	44.63	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-
80	19.7849	43.44	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
81	20.4404	1.42	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-
82	20.6515	4.94	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
83	21.2292	2.93	Rhodopin
84	21.8514	3.11	6,7-Epoxy pregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate
85	22.1180	0.97	Campesterol
86	22.4179	1.94	Stigmasterol

Table 5. DPPH radical scavenging antioxidant assay of medicinal plants

Concentration (µg/ml)	% RSA			
	Ascorbic acid	<i>Aloe barbadensis</i>	<i>Cereus hexagonus</i>	<i>Cissus quadrangularis</i>
100	20	46.5	62.8	21
200	30	58	67.1	34
300	44	65	72.9	44
400	60	68	74.5	76
500	82.5	82	81	88.5

The values are expressed in terms of (Mean ± Standard deviation)

Antioxidant activity

DPPH radical scavenging assay

All three plant extracts exhibited concentration-dependent DPPH radical scavenging activity (Table 5). At 500 µg/mL, *C. quadrangularis* showed the highest activity (88.5% RSA), followed by *C. hexagonus* (81.0%) and *A. barbadensis* (82.0%), approaching the standard ascorbic acid (82.5%). At lower concentrations (100–

300 µg/mL), *C. hexagonus* consistently outperformed the other extracts, with 62.8–72.9% RSA compared to 21–44% for *C. quadrangularis* and 46.5–65% for *A. barbadensis*. This suggests that *C. hexagonus* possesses potent hydrogen-donating or electron-transfer capacity at sub-maximal doses, likely attributable to its high content of unsaturated fatty acids (oleic, linoleic) and phenolic derivatives.

Table 6. Reducing power antioxidant assay of medicinal plants

Concentration (µg/ml)	% RSA			
	Ascorbic acid	<i>Aloe barbadensis</i>	<i>Cereus hexagonus</i>	<i>Cissus quadrangularis</i>
100	17	21.5	34.7	10
200	30	40	41.8	31
300	46	63.5	56.7	48
400	63	79	58	63.5
500	83.5	85	62.1	81.5

The values are expressed in terms of (Mean ± Standard deviation)

Reducing power assay

In the reducing power assay (Table 6), *A. barbadensis* demonstrated the strongest activity across all concentrations, reaching 85% RSA at 500 µg/mL, comparable to ascorbic acid (83.5%). *C. quadrangularis* and *C. hexagonus* showed moderate activity, with 81.5% and 62.1% RSA, respectively, at the highest concentration. The superior reducing power of *A. barbadensis* may be linked to its abundant phenolic compounds (e.g., 5-hydroxymethylfurfural, thymine, phenolic acids), glycosides, and sterols (campesterol, stigmasterol), which are effective electron donors in redox reactions.

DISCUSSION

The gas chromatography–mass spectrometry (GC-MS) profiles identified in this study highlight the profound chemical complexity and species-specific metabolomic signatures of *Aloe barbadensis* (Aladodo *et al.*, 2026), *Cereus hexagonus*, and *Cissus*

quadrangularis (Ghosh *et al.*, 2026) ethanolic extracts. Due to its superior sensitivity, resolution, and reproducibility, GC-MS serves as a benchmark platform for characterizing volatile and derivatized natural phytochemical constituents (Elghaffar *et al.*, 2022).

The extract demonstrated a high abundance of long-chain fatty acids, notably palmitic, oleic, linoleic, and stearic acids. This metabolic profile reflects the specialized lipid pathways characteristic of succulent xerophytes, where fatty acids and their derivatives fulfill vital roles in membrane stability, cuticular wax synthesis, and abiotic stress signaling. The detection of trimethylsilyl (TMS) derivatives corroborated the presence of core carboxylic acids and long-chain fatty alcohols. Notably, profiling of volatile fractions revealed benzoic acid as the predominant constituent (71.49%), alongside significant fractions of *n*-nonadecanol-1 (7.35%), 1-hexacosanol (5.26%), and

the triterpenoid hydrocarbon 2,6,10,15,19,23-pentamethyl-2,6,18,22-tetracosahexaene (3.91%) (Sharma *et al.*, 2026).

The identification of carbohydrate derivatives (glycerin, 3-deoxy-D-mannonic lactone, ethyl α -D-glucopyranoside), low-molecular-weight organic acids, and phenolic compounds (including 5-hydroxymethylfurfural and thymine) accords with the established presence of bioactive polysaccharides, anthraquinones, and chromones in *Aloe* parenchyma tissue.

Although the detection of compounds such as 1,3-propanediol, 2-(hydroxymethyl)-2-nitro-, and 9,12-octadecadienoyl chloride may represent extraction artifacts or derivatization byproducts, their co-occurrence with verified bioactive metabolites such (phytol, tocopherols, campesterol, stigmasterol) substantiates the broad-spectrum therapeutic and antioxidant potential of the gel matrix.

Among the evaluated taxa, *C. quadrangularis* exhibited the greatest structural diversity, characterized by high concentrations of pentacyclic triterpenoids (α -amyrin, β -amyrin, and lupenyl acetate), phytosterols (γ -sitosterol, campesterol and stigmasterol), and long-chain aliphatic alcohols such as 1-heptatriacontanol. This rich metabolomic profile is consistent with earlier reports attributing significant antimicrobial properties against avian pathogens to the diverse bioactive constituents identified via GC-MS in *C. quadrangularis* (Chenniappan *et al.*, 2020). Triterpenoids and phytosterols represent the primary bioactive drivers of anti-inflammatory and radical-modulating responses in *Cissus* species, where their co-occurrence with phenolics, flavonoids, and glycosidic moieties suggests synergistic biological efficacy.

The *in-vitro* antioxidant assays demonstrated distinct, concentration-dependent free-radical scavenging profiles across the three botanical extracts, reflecting their unique underlying chemistries:

In evaluating the *in vitro* antioxidant dynamics, each extract exhibited distinct mechanistic behaviors directly linked to its chemical makeup. *Cereus hexagonus* demonstrated superior DPPH radical-scavenging activity at lower concentrations, driven primarily by its abundance of benzoic acid, long-chain fatty acids, and aliphatic alcohols, which efficiently facilitate Hydrogen Atom Transfer (HAT). In contrast, *Aloe barbadensis* excelled in ferric reducing antioxidant power (FRAP), governed by its dense pool of phenolics, glycosides, anthraquinones, and tocopherols operating predominantly via Single Electron Transfer (SET) mechanisms. Meanwhile, *Cissus quadrangularis* achieved the highest overall DPPH radical clearance at peak concentration 500 μ g/mL, where its rich matrix of pentacyclic triterpenoids, phytosterols, and flavonoids acted synergistically across both HAT and SET pathways to drive multi-modal radical quenching.

The heightened DPPH radical scavenging observed for *C. hexagonus* at lower concentrations points toward a high-affinity Hydrogen Atom Transfer (HAT) mechanism, facilitated by phenolic hydroxyl groups and allylic hydrogens within unsaturated fatty acid chains.

Conversely, *A. barbadensis* demonstrated marked ferric reducing antioxidant potential, denoting strong electron-donating capacity driven by its rich pool of phenolic hydroxyls and reducing sugars. These observations align with findings by Hamid *et al.* (2017), which demonstrated that polar fractions of *Aloe* sp. mediate significant single-electron transfer (SET) reductions.

For *C. quadrangularis*, while moderate in reducing capacity, the extract achieved the highest total DPPH inhibition at 500 μ g/mL. As documented by Prasad *et al.* (2005), the capacity of *C. quadrangularis* extracts to donate hydrogen atoms and scavenge radical intermediates is heavily driven by its complementary flavonoid and phenolic pools. The peak radical scavenging capacity observed here underscores the cumulative, polyvalent interaction between

triterpenes, sterols, and polyphenols acting across both HAT and SET pathways.

The clear concentration dependence observed across all assays reaffirms the classical dose-responsive nature of plant-derived secondary metabolites. More broadly, the correlation between GC-MS metabolomic fingerprints and quantitative antioxidant performance supports the paradigm that targeted phytochemical classes specifically unsaturated fatty acids and simple aromatics in *Cereus*, glycosylated phenolics in *Aloe*, and pentacyclic triterpenoids in *Cissus* dictate distinct radical-neutralizing pathways. In the context of modern functional product formulation, delineating these species-specific bioactive profiles and their stability is essential for optimizing therapeutic dosing, enhancing shelf-life, and maximizing human health benefits (Naseem *et al.*, 2026).

The GC-MS analysis of the extract revealed a complex phytochemical profile dominated by fatty acids, phenolic compounds, terpenoids, and sterols, which collectively account for the observed antimicrobial and antioxidant activities. Among the identified constituents, n-hexadecanoic acid (palmitic acid), 9,12-octadecadienoic acid (linoleic acid, Z,Z-), and octadecanoic acid (stearic acid) were present in high relative abundance and are widely reported in the literature as potent antimicrobial agents capable of disrupting microbial cell membranes and interfering with essential enzymatic processes. These saturated and unsaturated long-chain fatty acids have been consistently associated with inhibitory effects against both Gram-positive and Gram-negative bacteria in GC-MS-based phytochemical studies, thereby supporting their role as primary contributors to the antimicrobial potential of the extract. In addition to fatty acids, phenolic compounds such as 5-hydroxymethylfurfural, catechol/benzenetriols, and 2-methoxy-4-vinylphenol were detected and are well known for their dual antimicrobial and antioxidant properties arising from their redox behaviour, hydrogen-donating capacity, and ability to interact with microbial membranes. The presence of

lipid-soluble antioxidants, particularly γ -tocopherol (vitamin E), along with phytol, stigmasterol, campesterol, and triterpenoids such as α -amyrin, β -amyrin, and lupeol derivatives, further strengthens the antioxidant profile of the extract, as these compounds are recognized for their radical-scavenging activity and protective effects against oxidative damage. Taken together, the high abundance of bioactive fatty acids, coupled with phenolics, tocopherols, sterols, and triterpenoids, suggests that the antimicrobial and antioxidant activities of the extract are likely due to the synergistic action of these compound classes rather than a single constituent, which is consistent with previous reports on plant-derived extracts analysed by GC-MS.

CONCLUSION

The present investigation demonstrates that ethanol extracts of *Aloe barbadensis*, *Cereus hexagonus* and *Cissus quadrangularis* are rich sources of bioactive phytoconstituents with notable antioxidant properties, as evidenced by qualitative screening and GC-MS profiling revealing diverse secondary metabolites including carbohydrates, flavonoids, glycosides, phenols, fatty acids, sterols, and triterpenoids. Antioxidant assays indicated concentration-dependent activity across all extracts, with *Cissus quadrangularis* displaying the highest DPPH radical scavenging activity at 500 $\mu\text{g/mL}$ (88.5% RSA), surpassing even the standard ascorbic acid, suggesting strong hydrogen-donating capacity likely due to its abundant triterpenoids and phenolics; *Cereus hexagonus* exhibited superior activity at lower concentrations (100–300 $\mu\text{g/mL}$), indicating potent antioxidant efficacy at sub-maximal doses, potentially attributable to its high unsaturated fatty acid content; and *Aloe barbadensis* demonstrated the strongest electron-donating ability in reducing power assay (85% RSA at 500 $\mu\text{g/mL}$), comparable to ascorbic acid, which may be linked to its rich phenolic and sterol composition. Collectively, these findings underscore the therapeutic potential of these plant extracts as natural antioxidants with distinct phytochemical profiles and antioxidant mechanisms suggesting complementary

applications: *C. quadrangularis* as a potent free radical scavenger, *C. hexagonus* for low-dose antioxidant efficacy, and *A. barbadensis* for redox-balancing applications, warranting further studies isolating specific bioactive compounds and evaluating their *in vivo* antioxidant and cytoprotective effects to validate their nutraceutical and pharmaceutical utility.

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