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Phytochemical profiling, GC–MS characterization and comparative antioxidant potential of ethanol extracts from *Euphorbia antiquorum* and *Opuntia ficus-indica***D. Ruffina*, L. Prince***P. G. & Research Department of Microbiology, Maruthupandiyar College, (Affiliated to Bharathidasan University, Trichy-24), Vallam- 613403, Thanjavur, Tamil Nadu, India***Key words:** Qualitative phytochemicals, Antioxidant activities, GC–MS, DPPH radical-scavenging, Reducing-power activities**Received:** August 24, 2026 **Accepted:** September 03, 2026 **Published:** September 12, 2026**DOI:** <https://dx.doi.org/10.12692/ijb/29.3.108-121>**ABSTRACT**

The present study evaluated the qualitative phytochemical composition of bioactive molecules by gas chromatography–mass spectrometry (GC–MS) profiles, and *in-vitro* antioxidant activities of ethanol extracts of *Euphorbia antiquorum* and *Opuntia ficus-indica*. Qualitative phytochemical screening revealed that both plant extracts contained carbohydrates, flavonoids, glycosides, phenols, proteins, and tannins were represented, whereas alkaloids, anthraquinones, steroids, and terpenoids were absent; saponins were detected exclusively in *O. ficus-indica*. GC–MS analysis were identified 20 compounds in each extract, with *E. antiquorum* dominated by 9-octadecenoic acid (Z)-, oxiranylmethyl ester (32.34%) and 2,4-di-tert-butylphenol (15.38%), while *O. ficus-indica* was characterized by β -sitosterol, TMS derivative (32.11%) and tetrapentacontane (27.77%). Antioxidant assays demonstrated concentration-dependent DPPH radical-scavenging and reducing-power activities for both plant extracts were determined. At 500 $\mu\text{g/mL}$, of *O. ficus-indica* exhibited marginally superior DPPH scavenging (79.1%) and reducing power (78.6) compared were also *E. antiquorum* (78.5% and 64.3% respectively), although both were less potent than the reference standard, ascorbic acid. These findings indicated that *O. ficus-indica* possesses a broader phytochemical profile and slightly enhanced antioxidant potential attributable to its higher sterol and long-chain hydrocarbon content, supporting its potential candidature as natural antioxidant activities for forthcoming generation.

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INTRODUCTION

The plants secondary metabolite was well recognized. The plant kingdom serves as a reservoir of potential pharmaceuticals, and in recent years, there has been a growing awareness regarding the value of medicinal plants. Plant-derived drugs are readily accessible, cost-effective, safe, and effective, with minimal side effects. The plants that have been chosen for medicinal purposes over millennia represent the most logical candidates for exploring the current quest for therapeutically potent new medications including anticancer agents (Dewick, 1996), antimicrobial substances (Phillipson and Wright, 1996), and antihepatotoxic agents. The World Health Organization (WHO) asserts that medicinal plants are the optimal source for a diverse array of drugs. Approximately 80% of individuals in developed nations utilize traditional medicine, which often incorporates compounds sourced from medicinal plants. Nonetheless, these plants require thorough investigation to enhance our understanding of their properties, safety, and efficacy (Arunkumar and Muthuselvam, 2009). Medicinal plants harbor various organic compounds that exert specific physiological effects on the human body including bioactive substances such as tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids (Edeoga *et al.*, 2005).

Diseases such as influenza, flu, dengue, malaria, tuberculosis, gonorrhea, pneumonia, septicemia, urinary tract infections, Japanese encephalitis, chikungunya, filariasis, and yellow fever, which are caused by viruses, bacteria, fungi, and parasites, are notoriously difficult to treat with antibiotics (Roopan *et al.*, 2013; Benelli, 2015). This challenge arises because the pathogens responsible for these infections are resilient and have developed various mechanisms to resist drug treatment (Odonkor and Addo, 2011). Globally, these diseases represent a significant health risk, as a substantial proportion of worldwide mortality can be linked to them, particularly in developing nations (Tallury *et al.*, 2010). Mosquito-borne diseases, in particular, are a major concern for public health due to their ability to

transmit diseases affecting over 700 million individuals annually. In India, mosquito-borne diseases are widespread across all states, facilitated by favorable ecological conditions (Patil *et al.*, 2012).

Dengue fever, which is caused by an RNA virus from the Flaviviridae family, is primarily transmitted by the *Aedes aegypti* mosquito, the main vector for dengue transmission worldwide (Khan *et al.*, 2010).

Euphorbia antiquorum is a succulent species that belongs to the Euphorbiaceae family, which is recognized as the largest and most varied family within the plant kingdom. This plant is distinguished by its three winged branchlets and stipular spines located on sinuate repend wings, with stipules having evolved into spines. The latex found in its branches contains compounds such as β -amyrin, cycloartenol, euphol, euphadienol, and euphorbol.

Additionally, the juice is rich in diterpene esters, specifically euphorbin. Both the stem-bark and latex are sources of triterpenoids, including taraxerol, taraxerone, friedelanol, and epifriedelanol, as well as euphol (Rajkuberan *et al.*, 2017; El-Ghani and Abdel-Khalik, 2006).

Consequently, this study highlights the antibacterial and larvicidal properties of latex extracts from *E. antiquorum*. The latex of plants has intrigued humanity for thousands of years, with significant research directed towards its bioactive properties for pharmaceutical uses (Rajkuberan *et al.*, 2015). Furthermore, the latex produced by the plant exhibits toxic effects against various insects and butterflies (Konno, 2011). Botanical insecticides are composed of chemical constituents such as alkaloids, terpenoids, saponins, tannins, phenolics, essential oils, and enzymes, which function as repellents, feeding deterrents, toxins, and growth regulators (Pavela and Govindarajan, 2017). Numerous studies have been conducted by researchers examining the efficacy of different plant extracts against various instar larval stages of mosquitoes (Govindarajan and Sivakumar, 2014; Sharma *et al.*, 2014).

Opuntia ficus-indica, widely recognized as prickly pear and classified within the Cactaceae family, is cultivated across various regions of Yemen for its utility in food, animal feed, and other products such as juice, jam, and cosmetics. This cultivation presents significant economic benefits (R'bia *et al.*, 2017; Al-Naqeb *et al.*, 2023). Native to Mexico, it was introduced to the Mediterranean area in the late 15th century and subsequently to North Africa in the late 16th century, from where it has proliferated globally (Ettalibi *et al.*, 2021; Ghanemi *et al.*, 2022). In the realm of traditional medicine, the fruit and young stems of *O. ficus-indica* have been utilized for the treatment of various ailments including hypertension, diabetes, allergies, asthma, burns, swelling, and nausea. Various components of the prickly pear plant, such as cladodes (pads), fruit, seeds, and seed oil, have been documented to possess health-promoting properties and biological activities (Belviranlı *et al.*, 2019; Kolniak-Ostek *et al.*, 2020).

These compounds are produced through the primary or more accurately, the secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically highly diverse substances with functions that remain largely unclear. They find extensive applications in human therapy, veterinary medicine, agriculture, scientific research, and numerous other fields (Vasu *et al.*, 2009). A significant number of phytochemicals from various chemical classes have demonstrated inhibitory effects against all types of microorganisms in vitro (Cowan, 1999). Plant-derived products have been integral to phytomedicines since ancient times. These can be sourced from barks, leaves, flowers, roots, fruits, and seeds (Cragg and Newman, 2001). Understanding the chemical constituents of plants is essential, as this knowledge is valuable for the synthesis of complex chemical compounds (Mojab *et al.*, 2003; Parekh and Chanda, 2008; Parekh and Chanda, 2009). In this study, qualitative phytochemical and GC-MS analyses were conducted on two plants, *Euphorbia antiquorum* and *Opuntia ficus-indica*, from the Northeastern region of India.

MATERIALS AND METHODS

Collection and preparation of plant materials

Healthy cladode samples of *Euphorbia antiquorum* and *Opuntia ficus-indica* were collected from Kolunji Ecological farm, Keeranur during February 2023.

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

Preparation of ethanol extracts

Approximately 20 g of powdered plant material was extracted with 250 mL of ethanol using a Soxhlet apparatus for 24 h. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 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 the phytochemical and antioxidant assays, the dried extracts were dissolved in ethanol and diluted to the required concentrations. The extraction yield was calculated using the following formula:

Extraction yield (%)

$$= \frac{\text{Weight of dried extract}}{\text{Weight of powdered sample}} \times 100$$

Qualitative phytochemical screening

The ethanol extracts were screened for the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, steroids, tannins and terpenoids (Harborne, 1967). Standard qualitative tests were performed using suitable reagents. Preliminary phytochemical screening commonly includes 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.

Alkaloid test

Approximately 1 mL of the extract was acidified with dilute hydrochloric acid and filtered. The filtrate was treated separately with Mayer's reagent and Dragendorff's reagent. Formation of a cream or reddish-brown precipitate indicated the presence of alkaloids.

Anthraquinone test

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

Carbohydrate test

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

Flavonoid test

The extract was mixed with a few drops of sodium hydroxide solution. Development of an intense yellow colour, which disappeared after the addition of dilute hydrochloric acid, was considered positive for flavonoids.

Glycoside test

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

Phenol test

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

Protein test

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

Saponin test

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

Steroid test

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

Tannin test

The extract was treated with ferric chloride solution. Formation of a blue, green or bluish-black colour indicated the presence of tannins.

Terpenoid test

The extract was mixed with chloroform, followed by the careful addition of concentrated sulphuric acid. Formation of a reddish-brown colour at the interface indicated the presence of terpenoids. The results were recorded as positive (+) when a characteristic colour change was observed and negative (–) when no visible reaction occurred.

DPPH radical-scavenging assay

The free-radical-scavenging activity of the ethanol extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Choi *et al.*, 2002). DPPH is a stable purple-coloured free radical that becomes reduced in the presence of antioxidant compounds, resulting in a decrease in absorbance. The decrease is commonly measured at approximately 517 nm.

A freshly prepared DPPH solution was prepared in ethanol at a concentration of 0.1 mM and protected from light. The 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. The reaction mixtures were vortexed and incubated in the dark at room temperature for 20–30 min.

The absorbance was measured at 517 nm using a UV–visible spectrophotometer. Ascorbic acid was used as the standard antioxidant. A reagent control containing DPPH solution without plant extract was also prepared. The assay was performed in triplicate, and the percentage of radical-scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where:

A_0 is the absorbance of the control.

A_1 is the absorbance of the extract or standard.

Higher percentage inhibition indicated greater DPPH radical-scavenging activity.

Reducing-power assay

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

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 incubation for 10 min, the absorbance was measured at 700 nm. Increased absorbance indicated greater reducing power. A similar ferric-reducing assay uses phosphate buffer and potassium ferricyanide followed by measurement of the coloured ferrous complex at 700 nm. Ascorbic acid was used as the reference standard.

Data analysis

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

RESULTS

Qualitative phytochemical analysis

The qualitative phytochemical constituents detected in the ethanol extracts of *Euphorbia antiquorum* and *Opuntia ficus-indica* appear in Table 1. Results showed that both extracts contained carbohydrates, flavonoids, glycosides, phenols, proteins and tannins. Alkaloids, anthraquinones and steroids were not detected in either extract.

Table 1. Qualitative phytochemical constituents of the ethanol extracts of *E. antiquorum* and *O. ficus-indica*

Phytocompounds	<i>Euphorbia antiquorum</i>	<i>Opuntia ficus indica</i>
Alkaloid	-	-
Anthraquinone	-	-
Carbohydrate	+	+
Flavonoid	+	+
Glycosides	+	+
Phenol	+	+
Protein	+	+
Saponin	-	+
Steroid	-	-
Tannin	+	+
Terpenoids	-	-

Table 2. GC-MS analysis of the ethanol extracts of *Euphorbia antiquorum*

Peak	R.Time	Area%	Name
1	22.199	15.38	2,4-Di-tert-butylphenol
2	26.473	2.74	1-Nonadecene
3	32.217	3.62	1-Nonadecene
4	36.170	3.61	1-Nonadecene
5	38.095	2.15	PHYTOL ISOMER
6	39.441	3.09	1-Hexacosanol
7	40.918	2.72	Glycidyl palmitate
8	41.674	1.89	O O'-BIPHENOL, 4,4',6,6'-TETRA-T-BUTY
9	42.098	1.63	PHENANTHRENE, 7-ETHENYL-1,2,3,4,4
10	42.339	2.17	Octacosanol
11	43.321	7.78	BICYCLO[10.1.0]TRIDEC-1-ENE
12	43.395	32.34	9-Octadecenoic acid (Z)-, oxiranylmethyl est
13	44.979	1.61	1-Hexacosanol
14	45.041	1.54	HEXATRIACONTANE
15	46.138	5.18	Tetrapentacontane
16	46.200	2.48	ELAIDINSAEURE, ISOPROPYLESTER
17	46.313	2.02	Tetrapentacontane
18	47.700	1.69	Hexadecanedinitrile
19	47.768	4.34	HEXATRIACONTANE
20	47.972	2.01	Squalene
		100.00	

Table 3. GC-MS analysis of the ethanol extracts of *Opuntia ficus indica*

Peak	R.Time	Area%	Name
1	22.176	5.93	2,4-Di-tert-butylphenol
2	26.459	1.03	1-Nonadecene
3	32.215	1.29	1-Nonadecene
4	36.169	1.35	1-Nonadecene
5	39.441	1.20	1-Heptacosanol
6	40.892	2.37	Glycidyl palmitate
7	41.674	0.64	O O'-BIPHENOL, 4,4',6,6'-TETRA-T-BUTY
8	42.338	0.91	1-Hexacosanol
9	42.721	0.68	9-Octadecenoic acid (Z)-, 2,3-dihydroxyprop
10	43.303	6.21	Linoleyl acetate
11	43.383	10.24	9-Octadecenoic acid (Z)-, oxiranylmethyl est
12	43.475	0.76	n-Propyl 9,12-hexadecadienoate
13	43.732	1.66	Myristic acid glycidyl ester
14	44.980	0.61	1-Hexacosanol
15	45.039	0.74	Dotriacontane
16	45.830	32.11	.beta.-Sitosterol, TMS derivative
17	46.142	27.77	Tetrapentacontane
18	46.320	2.60	Tetrapentacontane
19	47.771	1.36	HEXATRIACONTANE
20	47.973	0.52	Squalene
		100.00	

Saponins were detected only in the ethanol extract of *O. ficus-indica*, whereas they were absent in *E. antiquorum*. Terpenoids were not detected in either of the tested extracts. Overall, *O. ficus-indica* exhibited a slightly broader phytochemical profile due to the additional presence of saponins than *E. antiquorum*.

GC-MS analysis

The GC-MS chromatographic profiles of the ethanol extracts of *E. antiquorum* and *O. ficus-indica* are recorded in (Table 2 and 3) and (Fig. 1 and 2). A total of

20 compounds were identified in each plant extract based on their retention times and mass spectral matching. The identified compounds included phenolic compounds, alkenes, alcohols, esters, fatty-acid derivatives, sterol derivatives, hydrocarbons and terpenoid-related compounds.

Euphorbia antiquorum

The GC-MS analysis of the ethanol extract of *E. antiquorum* revealed 20 identifiable compounds, the major constituent was 9-octadecenoic acid (Z)-,

oxiranylmethyl ester, which represented 32.34% of the total chromatographic peak area. The second major compound was 2,4-di-tert-butylphenol, with an area percentage of 15.38%. Bicyclo[10.1.0]tridec-1-ene accounted for 7.78%, while tetrapentacontane contributed 5.18%.

Other identified compounds included hexatriacontane, 1-nonadecene, 1-hexacosanol, octacosanol, glycidyl palmitate, squalene, phytol isomer and elaidic acid isopropyl ester. The remaining compounds were present in relatively lower proportions, ranging from 1.54 to 4.34%.

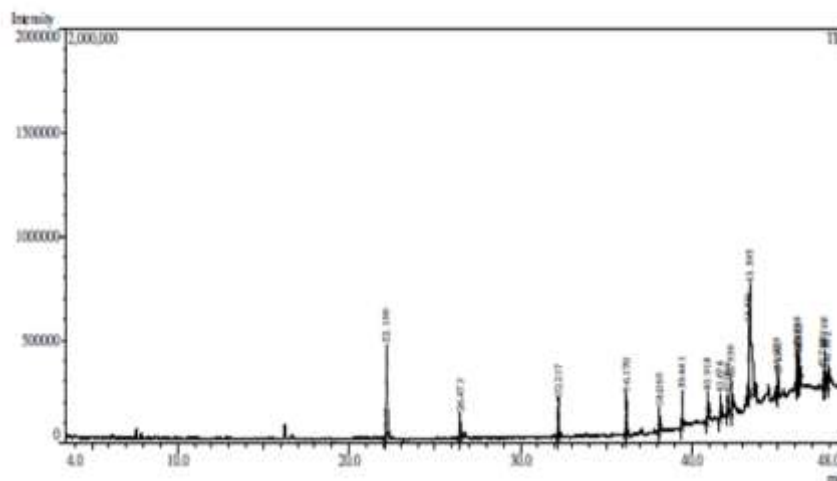


Fig. 1. GC-MS analysis of the ethanol extracts of *Euphorbia antiquorum*

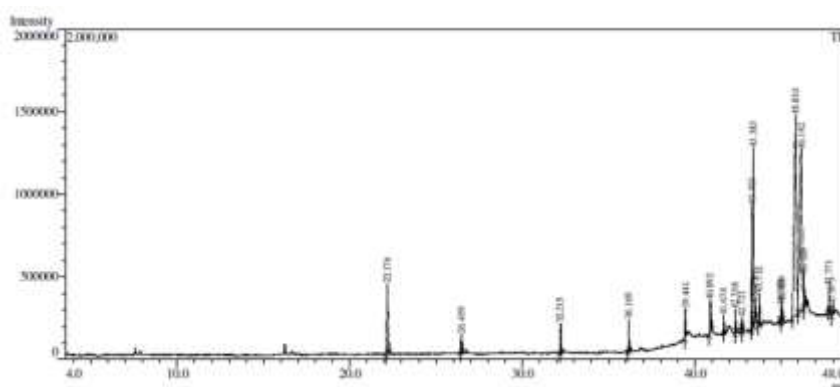


Fig. 2. GC-MS analysis of the ethanol extracts of *Opuntia ficus-indica*

The GC-MS profile indicated that the extract was predominantly composed of an oxirane-containing fatty-acid ester, a substituted phenolic compound and long-chain hydrocarbon derivatives. The presence of 2,4-di-tert-butylphenol is particularly notable because it constituted a comparatively large fraction of the detected extract.

Opuntia ficus-indica

The ethanol extract of *O. ficus-indica* also showed 20 identifiable compounds. The major compound was β -

sitosterol, TMS derivative, which represented 32.11% of the total peak area.

Tetrapentacontane was the second major constituent, accounting for 27.77%, followed by 9-octadecenoic acid (Z)-, oxiranylmethyl ester at 10.24%.

Other important compounds included 2,4-di-tert-butylphenol at 5.93%, linoleyl acetate at 6.21%, glycidyl palmitate at 2.37% and tetrapentacontane at an additional peak area of 2.60%. Minor constituents

included 1-nonadecene, 1-heptacosanol, 1-hexacosanol, n-propyl 9,12-hexadecadienoate, myristic acid glycidyl ester, dotriacontane, hexatriacontane and squalene.

Compared with *E. antiquorum*, the *O. ficus-indica* extract showed a higher relative contribution from sterol and long-chain hydrocarbon derivatives. The high abundance of β -sitosterol, TMS derivative, and tetrapentacontane characterized the GC–MS profile of this extract.

Comparative GC–MS profile

The two plant extracts were analysed with several compounds including 2,4-di-tert-butylphenol, 1-nonadecene, glycidyl palmitate, 9-octadecenoic acid (Z)-, oxiranylmethyl ester, long-chain hydrocarbons and squalene. However, their relative abundances differed considerably. The dominant compound in *E. antiquorum* was 9-octadecenoic acid (Z)-,

oxiranylmethyl ester, whereas β -sitosterol, TMS derivative was the principal compound in *O. ficus-indica*.

The area percentages obtained by GC–MS represent the relative abundance of the detected chromatographic peaks and should not be interpreted as absolute concentrations unless confirmed using appropriate analytical standards and quantitative calibration.

Antioxidant activity and reducing-power assay of *Euphorbia antiquorum*

The antioxidant activity of the ethanol extract of *E. antiquorum* was evaluated using DPPH radical-scavenging and reducing-power assays. In the DPPH assay, the percentage radical-scavenging activity of the extract increased progressively with concentration. The activity increased from 52.9% at 100 μ g/mL to 78.5% at 500 μ g/mL.

Table 4. DPPH radical scavenging antioxidant assay of medicinal plants

#	Concentration (μ g/ml)	% RSA		
		Ascorbic acid	<i>Euphorbia antiquorum</i>	<i>Opuntia ficus indica</i>
1	100	97.3	52.9	55.8
2	200	97.8	57.1	60.9
3	300	97.9	70.6	68.3
4	400	98.0	75.6	74.7
5	500	98.5	78.5	79.1

The values are expressed in terms of (Mean $\pm$ Standard deviation)

Ascorbic acid showed higher DPPH radical-scavenging activity for the tested concentrations, increasing from 97.3% at 100 μ g/mL to 98.5% at 500 μ g/mL. Although the activity of the plant extract was lower than that of the standard, the concentration-dependent increase demonstrated that the ethanol extract possessed considerable DPPH radical-scavenging potential (Table 4).

A similar concentration-dependent pattern was observed in the reducing-power assay (Table 5). The reducing-power value of the *E. antiquorum* extract increased from 5.7 at 100 μ g/mL to 64.3 at 500 μ g/mL. Ascorbic acid exhibited values ranging from 20 to 87 over the same concentration range. The progressive increase in the reducing response with increasing extract concentration indicated enhanced

electron-donating or reducing capacity at higher concentrations.

Antioxidant activity and reducing-power assay of *Opuntia ficus-indica*

The antioxidant activity of the ethanol extract of *O. ficus-indica* was also determined using DPPH radical-scavenging and reducing-power assays. The DPPH radical-scavenging activity increased from 55.8% at 100 μ g/mL to 79.1% at 500 μ g/mL. This gradual increase indicated a concentration-dependent enhancement of radical-scavenging activity (Table 4).

Ascorbic acid showed higher activity than the plant extract throughout the assay, with values increasing from 97.3% at 100 μ g/mL to 98.5% at 500 μ g/mL. Nevertheless, the extract displayed substantial

scavenging activity particularly at the highest concentration tested.

The reducing-power (Table 5) response of the *O. ficus-indica* extract increased from 18.2 at 100 µg/mL

to 78.6 at 500 µg/mL. Ascorbic acid produced values between 20 and 87. The increasing response observed with increasing extract concentration confirmed the concentration-dependent reducing capacity of the ethanol extract.

Table 5. Reducing-power assay of *Euphorbia antiquorum* and *Opuntia ficus indica*

#	Concentration (µg/ml)	% RSA		
		Ascorbic acid	<i>Euphorbia antiquorum</i>	<i>Opuntia ficus indica</i>
1	100	20	5.7	18.2
2	200	37	23.7	39.9
3	300	53	48.8	56.7
4	400	69	56.2	67.9
5	500	87	64.3	78.6

Comparison of antioxidant activity and reducing-power assays

Both plant extracts exhibited concentration-dependent antioxidant activity in the DPPH and reducing-power assays. At 500 µg/mL, *O. ficus-indica* showed slightly higher DPPH radical-scavenging activity than *E. antiquorum*, with values of 79.1 and 78.5%, respectively. Similarly, the reducing-power value of *O. ficus-indica* was higher at the maximum concentration, reaching 78.6 compared with 64.3 for *E. antiquorum*.

Overall, the ethanol extract of *O. ficus-indica* demonstrated slightly greater antioxidant activity than *E. antiquorum* under the tested conditions. However, both extracts showed lower activity than ascorbic acid, indicating that the extracts possessed antioxidant potential but were less effective than the reference standard at the tested concentrations.

DISCUSSION

The qualitative phytochemical analysis revealed that the ethanol extracts of *Euphorbia antiquorum* (Sakunpak *et al.*, 2014). and *Opuntia ficus-indica* contained carbohydrates, flavonoids, glycosides, phenols, proteins and tannins. Saponins were detected only in the extract of *O. ficus-indica*. Phytochemical analyses confirmed the presence of coumarins, steroids, and terpenoids in both cladode and fruit extracts, whereas tannins were not detected (Santana *et al.*, 2026). Alkaloids, anthraquinones,

steroids and terpenoids were not detected in either extract (Sivaraj *et al.*, 2011). The presence of phenols, flavonoids and tannins is important because these phytochemicals are commonly associated with hydrogen-donating and electron-donating properties, which may contribute to the antioxidant activity of plant extracts (Sun *et al.*, 2023). Phytochemical screening of bioactive plant extracts has revealed the presence of alkaloids, tannins and flavonoids in Cactus (*Opuntia ficus-indica*), flavonoids and tannins have been linked to antibacterial activity and antidiarrheal activity (Sayyah *et al.*, 2004; Abd El-Rahman *et al.*, 2012).

The difference in saponin content between the two plants may be related to their distinct chemical composition. It may also be influenced by the plant part used, maturity, geographical conditions and extraction procedure. The absence of a phytochemical group in a qualitative test does not necessarily confirm its complete absence, as the compound may be present below the detection limit or may not have been efficiently extracted by ethanol.

GC–MS analysis identified 20 bioactive compounds in each ethanol extract. In *E. antiquorum*, 9-octadecenoic acid (Z)-, oxiranylmethyl ester was the predominant compound, representing 32.34% of the total peak area. The second major compound was 2,4-di-tert-butylphenol, which accounted for 15.38%. Other compounds included bicyclo[10.1.0]tridec-1-ene, tetrapentacontane, hexatriacontane, 1-

hexacosanol, octacosanol, squalene and different ester derivatives. These bioactive components which may possess several pharmacological properties. Squalene is one among the compounds of the present study. Squalene possesses chemo-preventive activity against the colon carcinogenesis (Rao *et al.*, 1998).

In *O. ficus-indica*, β -sitosterol, TMS derivative, was the major compound with an area percentage of 32.11%, followed by tetrapentacontane at 27.77%. 9-Octadecenoic acid (Z)-, oxiranylmethyl ester and linoleyl acetate contributed 10.24 and 6.21%, found to be recorded respectively. The presence of 2,4-di-tert-butylphenol, fatty-acid esters, sterol derivatives and squalene suggested that the extract possesses a chemically diverse composition. Similar studies have reported phenolic, flavonoid and other antioxidant constituents in *O. ficus-indica* extracts. Therefore, phenolic compounds of prickly pear peels are good electron donors and could terminate the radical chain reaction by converting free radical to more stable products (Abou-Elella and Ali, 2014).

The DPPH assay showed a concentration-dependent increase in radical-scavenging activity in both plant extracts. The activity of *E. antiquorum* increased from 52.9% at 100 $\mu\text{g/mL}$ to 78.5% at 500 $\mu\text{g/mL}$. Similarly, *O. ficus-indica* showed an increase from 55.8% to 79.1% over the same concentration range. DPPH and ABTS scavenging assays are widely utilized to evaluate the free radical scavenging ability in vitro (Gulcin, 2020). This progressive increase indicated that the extracts contained compounds capable of neutralizing DPPH free radicals and that their activity increased with the quantity of extract used. Antioxidant activities were reported in 91% of studies (Bishikwabo *et al.*, 2026).

Ascorbic acid showed greater DPPH activity than both plant extracts, with a maximum value of 98.5% at 500 $\mu\text{g/mL}$. The higher activity of ascorbic acid may be attributed to its purified nature and strong reducing capacity, whereas the plant extracts contain a mixture of compounds with different levels of activity. Nevertheless, both extracts exhibited

considerable radical-scavenging activity particularly at 400 and 500 $\mu\text{g/mL}$.

The reducing-power assay also demonstrated an increase in activity with increasing concentration. The reducing-power value of *E. antiquorum* increased from 20 at 100 $\mu\text{g/mL}$ to 64.3 at 500 $\mu\text{g/mL}$, while *O. ficus-indica* increased from 18.2 to 78.6. Our findings support existing literature which identified *Euphorbia hirta*, *Euphorbia heterophylla*, and *Euphorbia convolvuloides* as having good antioxidant performance in FRAP and CUPRAC assays (Mahomoodally *et al.*, 2020). The higher value obtained for *O. ficus-indica* at 500 $\mu\text{g/mL}$ indicated comparatively greater electron-donating ability under the tested conditions. The results of both antioxidant assays suggested that *O. ficus-indica* had slightly higher antioxidant potential than *E. antiquorum*. The antioxidant effects are due to the major flavonoids encountered in cactus fruits. Flavonoids are more efficient antioxidants than vitamins, since phenolic compounds are able to delay pro-oxidative effects in proteins, DNA, and lipids by the generation of stable radicals (Aires *et al.*, 2004).

The antioxidant activity of the extracts may be due to the combined action of phenols, flavonoids, tannins, saponins and GC-MS-detected compounds such as 2,4-di-tert-butylphenol and β -sitosterol derivatives. Antioxidant activity was confirmed by DPPH and ABTS assays (Pansera *et al.*, 2026). However, the antioxidant effect cannot be assigned to a single compound because crude plant extracts contain several constituents that may act individually or synergistically. In addition, the GC-MS area percentage indicates the relative abundance of detected compounds and does not represent their exact concentration or direct biological activity.

The GC-MS analysis revealed a chemically diverse profile comprising phenolic compounds, terpenoids, sterols, long-chain fatty alcohols, fatty-acid esters and hydrocarbons. Among the identified constituents, 2,4-di-tert-butylphenol may be considered a major contributor to the antimicrobial and antioxidant

properties of the extract because its phenolic hydroxyl group can participate in free-radical scavenging and interact with microbial membranes and cellular proteins. This compound has previously demonstrated antifungal activity against *Aspergillus*, *Fusarium* and *Penicillium* species, in addition to antibacterial activity against selected pathogens. Squalene, phytol isomer and β -sitosterol may further enhance the biological activity through membrane interaction, lipid-phase antioxidant protection and modulation of oxidative stress. The long-chain alcohols, including 1-heptacosanol, 1-hexacosanol and octacosanol, may contribute to antimicrobial activity by disturbing microbial membrane integrity, whereas the fatty-acid esters may exert moderate membrane-associated effects. In contrast, long-chain alkanes such as dotriacontane, hexatriacontane and tetrapentacontane are likely to have mainly supportive or synergistic roles and are not expected to be strong direct antioxidants. Therefore, the observed antimicrobial and antioxidant activities are probably due to the cumulative and synergistic effects of several compounds rather than the action of a single metabolite (Varsha *et al.*, 2015; Rouvier *et al.*, 2024; Togashi *et al.*, 2007; Luhata and Usuki, 2021).

The compound annotations should be treated as tentative, particularly where names are truncated, isomers are indicated or TMS derivatives are reported. Antimicrobial activity should be verified using MIC/MBC or MIC/MFC assays, while antioxidant potential should be confirmed using complementary methods such as DPPH, ABTS, FRAP and metal-chelating assays.

Overall, the results demonstrated that both medicinal plants possess important phytochemical constituents and significant in-vitro antioxidant activity. *O. ficus-indica* showed slightly better DPPH radical-scavenging and reducing-power activity than *E. antiquorum*.

CONCLUSION

The comparative analysis of *Euphorbia antiquorum* and *Opuntia ficus-indica* ethanol extracts revealed

distinct phytochemical profiles that correlated with their antioxidant activities. Both extracts contained bioactive phenolics, flavonoids and long-chain hydrocarbons contributing to free radical scavenging. The exclusive presence of saponins and higher sterol content in *O. ficus-indica* likely accounts for its slightly superior DPPH and reducing-power activities when compared to *E. antiquorum*.

Although both extracts demonstrated significant concentration dependent antioxidant effects, their activities were lower than ascorbic acid, suggesting potential for further optimization through fractionation or synergistic formulations. The dominance of β -sitosterol and long-chain hydrocarbons in *O. ficus-indica* positions it as a more promising candidate for natural antioxidant applications in pharmaceutical and nutraceutical industries. However evaluating additional biological activities to fully exploited their therapeutic potential.

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