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INVESTIGATING THE QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF ACANTHOCEREUS TETRAGONUS (CACTACEAE FAMILY) ALONG WITH THE ANTIOXIDANT ACTIVITY

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ABSTRACT

Acanthocereus tetragonus is a cactus plant belonging to family Cactaceae. Literature suggests that cactus plants are known for their anti-inflammatory, anti-ulcer, anti-diabetic, anti-obesity, and anti-cancer properties. Thus, cactus plants have a wide range of therapeutic properties and have been used for centuries by indigenous people for their medicinal benefits. While more research is needed to fully understand the mechanisms behind these therapeutic effects, the use of cactus plants in traditional medicine is a promising avenue for future research and development of new therapeutics. Therefore, the present study aims to evaluate the qualitative and quantitative phytochemicals and antioxidant property of Acanthocereus tetragonus plant. The study reports the total phenolic and flavonoid content of both hydro-methanolic and ethyl acetate extracts, using the Folin-Ciocalteau and aluminium chloride method respectively. The hydro-methanolic extract exhibited higher total phenolic content, while the ethyl acetate extract had a higher total flavonoid content. Antioxidant activity was also measured using DPPH and FRAP assays, and the hydro-methanolic extract showed comparatively better free radical scavenging activity than ethyl acetate extract. However, the study highlights the importance of quantitative phytochemical analysis for understanding the chemical composition and their potential benefits for human health. The results also suggest that A. tetragonus extracts have significant antioxidant activity and can be explored further for their potential medicinal and therapeutic uses.

Keywords: *Acanthocereus tetragonus, cactus, phenol, flavonoids, antioxidant, phytochemicals*

INTRODUCTION

The use of plants for therapeutic purposes has a rich history in traditional medicine practices around the world. Plants have been used for medicinal purposes since ancient times, and many traditional healing practices around the world still rely on plant-based remedies (Balick & Cox, 2020). Plants contain a wide range of natural compounds such as alkaloids, flavonoids, and terpenoids, which have various biological activities and can be used for treating or preventing diseases. Many plant-based remedies have been shown to have efficacy in modern scientific studies, and ongoing research continues to explore the potential health benefits of plant compounds.

The Cactaceae family, commonly known as the cactus family, is a diverse and unique group of succulent plants that are found throughout the Americas. These plants have adapted to survive in some of the harshest environments on Earth, including deserts and arid regions. Cactaceae plants are characterized by their succulent stems and spiny leaves, which have evolved to store water and protect the plant from herbivores. They have also developed specialized root systems that allow them to absorb water and nutrients from the soil, even in extremely dry conditions. The Cactaceae family is divided into several subfamilies and hundreds of genera, with over 2,000 known species (Anderson, 2001). These plants have a wide range of uses, from ornamental plants in gardens and homes to medicinal and culinary applications.

Cactus species contain a variety of phytochemicals, which are natural compounds produced by plants that have various biological functions. The most well-known phytochemicals found in cacti are alkaloids, phenols, flavonoids, and betalains. Some cactus species such as prickly pear cactus (Opuntia spp.) contain flavonoids such as quercetin and kaempferol, which have been shown to have potential health benefits such as reducing inflammation and improving cardiovascular health (Tesoriere et al., 2004). In addition to these phytochemicals, cactus species also contain a range of other compounds such as polysaccharides, terpenoids, and sterols, which have various biological functions. These

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compounds may have potential therapeutic uses such as wound healing, anti-inflammatory effects, and immune system stimulation.

Acanthocereus tetragonus, also known as the triangle cactus, is a species of cactus native to Central and South America. The plant has triangular stems with sharp, needle-like spines along the edges. *Acanthocereus tetragonus* is commonly grown as an ornamental plant due to its interesting shape and fast growth. It is also used in traditional medicine in some regions to treat a variety of ailments, including digestive issues and skin problems. In addition to its medicinal uses, *Acanthocereus tetragonus* is also used as a food source in some regions. Overall, *Acanthocereus tetragonus* is a unique and versatile plant that has both ornamental and medicinal uses. Its fast growth and interesting shape make it a popular choice for home gardens and landscaping projects, while its fruit and potential health benefits make it a valuable resource in traditional medicine and food production (Niyaz & Ravikumar, 2022).

However, more research is needed to fully understand the other biological activities and potential therapeutic uses of this plant. The current study attempts to do so by evaluating the qualitative and quantitative phytochemical analysis and antioxidant potential of this plant.

MATERIAL & METHODOLOGY

Plant Collection

In this study, the cladodes of *Acanthocereus tetragonus* were obtained from Chotila town in the Surendranagar district of Gujarat, India, located near Rajkot. The collected sample underwent a thorough washing with distilled water to effectively remove dust and small spines. Large spines present on the stem/cladodes were eliminated using a sterile blade. The stems were then sectioned and subjected to oven drying for a period of 30 hours at a temperature of 45°C. Following this, the dried samples were pulverized into a fine powder using a mixer and stored at ambient temperature for subsequent analysis.

Plant extract preparation

The preparation of extracts from *Acanthocereus tetragonus* cladodes was carried out using the hot Soxhlet extraction method. Dry powder, weighing 20 g, was placed in the extraction thimble, and 200 ml of solvent was used to initiate the extraction process. Two solvents of different polarities, namely hydro-methanolic (30:70) and ethyl acetate, were used separately to produce two distinct cactus extracts. Subsequently, the extracts were filtered with Whatman filter paper no. 1, and the solvents were eliminated using a rotary evaporator. The resultant extracts were then preserved at a temperature of 4 °C for further investigation. The extraction yield of all the prepared extracts was computed using the following formula:

Plant Yield (%) = (Weight of crude extract x 100) / Weight of dry powder used.

Preliminary phytochemical analysis

Different protocols were used to test the presence and absence of various phytochemicals present in the two extracts of *A. tetragonus* plant. The following tests were followed as explained by Shah et al., (2021).

Alkaloids tests

- Mayer's test: Add a few drops of Mayer's reagent (potassium mercuric iodide) to the plant extract or solution. Formation of a white or cream-colored precipitate indicates the presence of alkaloids.
- Wagner's test: Add a few drops of Wagner's reagent (iodine and potassium iodide solution) to the plant extract or solution. Formation of a brownish-red precipitate suggests the alkaloids presence.
- Dragendorff's test: add a few drops of Dragendorff's reagent (potassium bismuth iodide) to the plant extract or solution. Formation of an orange or red precipitate indicates the presence of alkaloids.
- Hager's test: Add a few drops of Hager's reagent (saturated picric acid solution) to the plant extract or solution. Formation of a yellow precipitate indicates the presence of alkaloids.

Phenols tests

- Ferric chloride test: involves adding a few drops of ferric chloride solution to the plant extract. The presence of phenols is indicated by the formation of a bluish-black or greenish-black coloration. This coloration results from the formation of a complex between the ferric ion and the phenolic group in the plant extract.
- Lead acetate test: involves adding a few drops of lead acetate solution to the plant extract. The presence of phenols is indicated by the formation of a yellow precipitate. This reaction occurs because lead acetate reacts with the phenolic group to form lead phenolate, which is insoluble in water and precipitates out.
- Folin-Ciocalteu Test: This test involves adding Folin-Ciocalteu reagent to the plant extract, followed by sodium carbonate solution. Phenols present in the extract will produce a blue color, which can be measured using a spectrophotometer.

Flavonoids tests

- Lead Acetate Test: Add lead acetate solution to the plant extract and the flavonoids present in the extract will produce a yellow precipitate.
- Ammonia Test: In this test, a few drops of dilute ammonia solution are added to the plant extract. Flavonoids present in the extract will produce a yellow coloration.
- Aluminum Chloride Test: Add a few drops of 5% aluminum chloride solution to the plant extract and flavonoids present in the extract will produce a yellow color.

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Terpenoid tests

- Salkowski test: involves adding concentrated sulfuric acid to a plant extract and observing the formation of a reddish-brown color. This color indicates the presence of terpenoids in the sample.
- Borntrager's test: involves treating the plant extract with a mixture of chloroform and concentrated sulfuric acid. The resulting mixture is then shaken and allowed to settle, and the upper layer is observed for the formation of a pink, purple or red color. This indicates the terpenoids presence in the sample.

Glycosides tests

- Keller-Killiani Test: This test involves the addition of a few drops of glacial acetic acid, followed by the addition of a solution of ferric chloride. A reddish-brown ring at the junction of the two layers indicates the presence of cardiac glycosides.
- Legal's Test: This test involves the addition of a few drops of a solution of digitoxin and glacial acetic acid to the plant extract. The mixture is then heated with a few drops of concentrated sulfuric acid. A reddish-brown coloration at the junction of the two layers indicates the presence of cardiac glycosides.

Saponin tests

- One common test involves the use of foam formation, which is observed when an aqueous solution of the plant extract is shaken vigorously. The formation of a persistent, stable foam indicates the presence of saponins.

Quantitative phytochemical screening

Total phenol content

In this study, the measurement of the total phenolic content of Acanthocereus tetragonus was conducted using the Folin-Ciocalteau method, with modifications made to the protocol as described by Rutuba et al. (2021). A standard solution of gallic acid or a sample solution with a concentration of 1 mg/ml (500 µl) was prepared and diluted with 10 ml of distilled water. Additionally, 500 µl of Folin-Ciocalteau reagent was added and the solution was incubated for 5 minutes. Next, 20% sodium carbonate (2 ml) was added and the volume was adjusted to 25 ml with distilled water. The mixture solution was thoroughly mixed and allowed to incubate in the dark for 30 minutes. Using a spectrophotometer, the absorbance was measured at 765 nm. With the help of the regression equation derived from a standard calibration curve, the total phenolic content was calculated and marked as milligrams of gallic acid equivalent per gram of sample (mg GAE/g of sample).

Total flavonoid content

In the present study, we employed a modified procedure based on the method outlined by Shah et al. (2021) to determine the total flavonoid content of Acanthocereus tetragonus using an aluminium chloride colorimetric assay. To do so, we added 500 µl of sample to test tubes along with 10% aluminium chloride (50 µl) and 1 M potassium acetate (50 µl). The resulting mixture was diluted with 10 ml of distilled water, stirred, and then incubated for 30 minutes. The spectrophotometer was used to record the readings at 415 nm. We utilized quercetin with varying concentrations as a standard, and followed the same protocol. The total flavonoid content was obtained using a regression equation from the standard curve. The results were indicated as milligrams of quercetin equivalent per gram of sample (mg QE/g of sample).

Antioxidant activity

DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for measuring the antioxidant capacity of plants. In this protocol, a solution of DPPH is added to a plant extract or compound, and the absorbance is measured at a specific wavelength of 517 nm. As the antioxidant compound in the plant extract reacts with the DPPH, the absorbance decreases. The degree of decrease in absorbance is proportional to the antioxidant capacity of the plant extract (Zeghad et al., 2019). The protocol involves preparing a stock solution of DPPH, diluting the stock solution to a working concentration, preparing plant extracts, mixing the extracts with 2 ml of DPPH solution, and measuring the absorbance. This method is a quick and reliable way to assess the antioxidant potential of plant extracts.

% Inhibition = $[(A_c - A_s)/A_c] \times 100$

Where A_c is the absorbance of the DPPH solution without the plant sample, and A_s is the absorbance of the DPPH solution with the plant sample.

FRAP assay

Acanthocereus tetragonus cladode extracts were subjected to evaluation of their antioxidant capacity using the Ferric Reducing Antioxidant Power (FRAP) assay. The preparation of FRAP reagent was done by combining TPTZ solution, $FeCl_3$ solution, and acetate buffer in a 1:1:10 ratio. Minor modifications were made to the standard protocol, wherein 500 µl of the sample, 10 ml of distilled water and 4 ml of FRAP reagent were mixed. The spectrophotometer readings were taken at 593 nm, and $FeSO_4.7H_2O$ was used as the standard to generate the standard curve at various concentrations. The results were expressed as milligrams of ferrous equivalent Fe (II) per gram of sample. (Wu et al., 2022).

Statistical analysis

To eliminate any chance of error, the tests were repeated three times. The findings of each experiment were displayed as mean± standard deviation. The ANOVA procedure was used to identify significant differences in the means, and Pearson's correlation coefficient was used to calculate the linear connection between the test variables. The Graph Pad Prism Software, Version 8, was used for all the statistical analysis.

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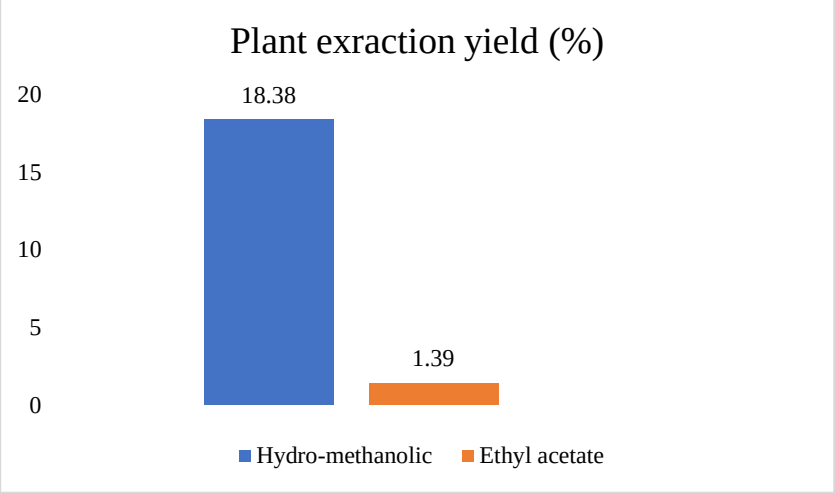
RESULTS & DISCUSSION

Plant extraction yield

Plant extraction yield refers to the amount of desirable compounds that can be obtained from a given quantity of plant material using a specific extraction method. The yield is influenced by several factors, including the type of plant, the plant part being used, the solvent used for extraction, the extraction method employed, and the duration of extraction (Nuzul et al., 2022). In the current study, hydro-methanolic solvent in a ratio of 30:70 and ethyl acetate solvents were employed to extract the bioactive components from the *Acanthocereus tetragonus*. The percentage yield obtained by the hydro-methanolic extract was 18.38% and the ethyl acetate extract displayed 1.39% plant extraction yield. The results of the current study suggests that the higher amount of phytochemicals are extracted by hydro-methanolic solvent as compared to the ethyl acetate solvent due to its high polar nature (Senguttuvan et al., 2014).

Fig-1 Plant extraction yield of hydro-methanolic & ethyl acetate extracts of *A. tetragonus* plant



Results for preliminary phytochemical analysis

Preliminary phytochemical screening is a common practice used to identify the presence of various phytochemicals or plant-derived compounds in plant extracts. The screening typically involves the use of several tests to detect the presence of various classes of compounds such as alkaloids, flavonoids, saponins, terpenoids, glycosides and phenolic compounds. The hydro-methanolic and ethyl acetate extracts of A. tetragonus plant was screened for phytochemical analysis and following results were obtained as displayed in Table-1.

Table-1 Preliminary phytochemical screening of hydro-methanolic and ethyl acetate extracts of *A. tetragonus*

Phytochemical	Test	Solvent	
		Hydro-MeOH (30:70)	Ethyl acetate
Alkaloids	Mayer's test	+	-
	Wagner's test	-	+
	Hager's test	+	-
	Dragendorff's test	-	+
Glycosides	Keller- killiani test	+	+
	Legal's test	-	-
Phenols	Ferric chloride test	+	-
	Lead acetate test	+	-
	Folin-Ciocalteu test	+	+
Flavonoids	Aluminium chloride test	+	-
	Ammonia test	+	-
	Lead acetate test	-	+
Saponins	Foaming test	+	-
Terpenoids	Salkowski test	+	-
	Borntrager's test	-	+
Cardiac glycosides	Sodium nitroprusside test	-	+

(+ sign & - sign indicates the presence and absence of the phytochemicals in the extracts)

Results for quantitative phytochemical analysis

Quantitative phytochemical analysis is an essential tool for understanding the chemical composition of plants and their potential uses in medicine, agriculture, and food safety. Phytochemicals are natural compounds that are responsible for the biological activity of plants, such as antioxidant, anti-inflammatory, and anti-cancer effects. Quantitative analysis of these compounds is important for quality control purposes, such as ensuring that plant-based products are consistent in their phytochemical content. It is also useful for identifying and quantifying potentially harmful compounds in plants,

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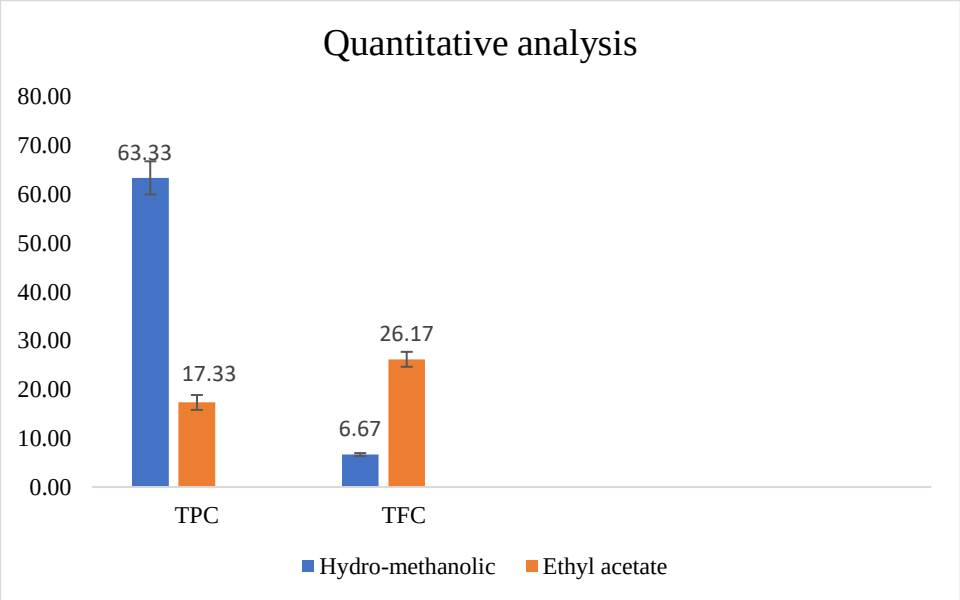
which can play a role in food safety (Santhi & Sengottuvel, 2016). By determining the concentration of these compounds, their medicinal properties can be established and appropriate dosages can be determined for therapeutic effect. Overall, quantitative phytochemical analysis is a crucial tool in understanding the chemical makeup of plants and their potential benefits. In the present work, the total phenolic and flavonoid content of both the extracts- hydro-methanolic and ethyl acetate extracts of *Acanthocereus tetragonus* plant was calculated.

Phenolic compounds are natural organic compounds that are widely distributed in plants, and they are known for their diverse biological activities. These compounds are characterized by the presence of one or more hydroxyl groups attached to an aromatic ring. The phenolic content of plants can vary depending on several factors such as the species, the environmental conditions, and the stage of development. Phenolic compounds have attracted significant attention due to their potential health benefits, which include antioxidant, anti-inflammatory, and anti-cancer properties. As a result, there has been a growing interest in the study of phenolic content in plants for medicinal and therapeutic purposes. The total phenolic content of *Acanthocereus tetragonus* was studied using Folin- Ciocalteau method in the present study. Among both the extracts studied, hydro-methanolic extract (63.33±3.40 mg GAE/g of sample) of *A. tetragonus* showed the remarkable total phenolic content than the ethyl acetate extract (17.33±1.53 mg GAE/ g of sample). Both the extracts exhibited significant differences in their total phenolic content ($P<0.05$). The total phenolic content showed strong positive correlation with the antioxidant FRAP values of *A. tetragonus* extracts. Cornejo-Campos et al., (2022) reported in their study that the crude extracts of A. tetragonus displayed 40.79 ± 1.00 µg GAE per mg of DW total phenolic content and 27.52 ± 1.36 µg GAE per mg of DW in cooked *A. tetragonus sample*. This values still proves that the total phenolic content was highest in the hydro-methanolic extract revealed in the present study than the values reported by others.

Flavonoids are a group of secondary metabolites found in plants, which are responsible for a wide range of biological activities. Flavonoids can be classified into several subgroups based on their chemical structure, including flavones, flavonols, flavanones, isoflavones, and anthocyanins, among others. They are known for their antioxidant, anti-inflammatory, and anti-cancer properties, and are believed to have a variety of health benefits for humans. In the current study, the total flavonoid content of ethyl acetate extract was reported more 26.17±1.53 mg QE/g of sample as compared to the hydro-methanolic extract (6.67±0.29 mg QE/ g of sample) of *A. tetragonus* plant. This shows the variation ($P<0.05$) in the total flavonoid content among both the extracts. Bakari et al., (2017) in their work reported the total flavonoid content of ethyl acetate extract of cladode of *Opuntia ficus indica* to be 69.1 ± 1.3 mg QE/g. This suggests the higher flavonoid content in *O. ficus indica* as compared to *A. tetragonus*. TFC is often used as a measure of the antioxidant capacity of a plant extract, as flavonoids are known to have potent antioxidant properties. The results suggest that the ethyl acetate extract showed the highest total flavonoid content while the hydro-methanolic extract exhibited the prominent total phenolic content in them. The total flavonoid content of ethyl acetate extract displayed significant correlation with the FRAP values of ethyl acetate extract of *A. tetragonus* plant. However, the determination of TFC provides valuable information for assessing the potential biological activity and nutritional value of plant extracts and products.

Fig-2 Quantitative phytochemical analysis of different extracts of *A. tetragonus* plant



Results for antioxidant activity

Plants possess various compounds that exhibit antioxidant activity, which refers to their ability to neutralize or scavenge free radicals and other reactive oxygen species (ROS) that can cause cellular damage and contribute to the development of various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. The antioxidant activity of plants is primarily attributed to the presence of phytochemicals such as flavonoids, phenolic acids, carotenoids, and tocopherols, among others. These compounds can act as direct antioxidants by donating electrons or hydrogen atoms

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to neutralize free radicals or indirectly by upregulating the expression of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Patel et al., 2010).

Moreover, some plant compounds exhibit metal-chelating activity, which prevents metal ions from catalyzing the formation of ROS. Plant antioxidants can also modulate various signaling pathways and gene expression to mitigate oxidative stress and inflammation, thereby protecting cellular components such as DNA, proteins, and lipids from damage. The free radical scavenging activity of hydro-methanolic extract (IC_{50}- 290.36 ± 1.40 µg/ml) of *A. tetragonus* was comparatively good than the ethyl acetate extract (IC_{50}- 312.27 ± 0.64 µg/ml) in the present study. While, the DPPH value of three different cultivars of *Opuntia ficus indica* was found to be around 127000 trolox equivalents/kg DW (Astello-Garcia et al., 2015) and 45.05 mg Trolox equivalents/ kg FW as reported by De Santiago et al., (2018). However, the results were comparable with the antioxidant activity of *A. tetragonus* plant as reported in the present study.

Along with the DPPH activity, FRAP assay was also conducted to evaluate the antioxidant power of *A. tetragonus* cladodes. The highest antioxidant activity of *A. tetragonus* using FRAP assay was reported by hydro-methanolic extract as compared to the ethyl acetate extract which was 149.67 ± 5.62 mg $FeSO_4$ equivalent/ g and 79.00 ± 4.77 mg $FeSO_4$ equivalent/ g respectively. Both the extracts showed prominent changes in their FRAP values ($P<0.05$). Bakari et al., (2017) in their work stated that the ethyl acetate extract (EC_{50} = 125 ± 0.4 µg/mL) of *Opuntia ficus indica* cladodes displayed highest antioxidant potential than its hexane (EC_{50} = 820 ± 0.4 µg/mL), ethanol (EC_{50} = 620 ± 0.2 µg/mL), acetone (EC_{50} = 325 ± 0.1 µg/mL) and dichloromethane (EC_{50} = 825 ± 0.1 µg/mL) extracts. Bezerril et al., (2021) gave FRAP value of *Pilosocereus gounellei* cladode to be 1912.95 µM of TEAC/100 g which was comparatively higher than its DPPH value (572.96 µM of TEAC/100 g). Due to the flavonoid structure, the amount of flavonoids in food may have a significant antioxidant capacity. Additionally, the association between phenolic compound concentration and antioxidant activity as determined by the DPPH and FRAP methods.

Overall, the antioxidant activity of plants is a complex interplay of multiple mechanisms and compounds that work together to maintain cellular homeostasis and prevent oxidative damage. Studies have shown that regular consumption of plant-based foods can provide significant health benefits by reducing the risk of chronic diseases associated with oxidative stress.

Table-2 Antioxidant potential of hydro-methanolic and ethyl acetate extracts of *A. tetragonus*

Antioxidant activity of *Acanthocereus tetragonus*		
Solvents	**DPPH values (IC_{50} µg/ml)**	**FRAP values (mg $FeSO_4$ equivalent/ g)**
Hydro-MeOH extract (30:70)	290.36 ± 1.40	149.67 ± 5.62
Ethyl acetate extract	312.27 ± 0.64	79.00 ± 4.77

CONCLUSION

The current study quantified the total phenolic and flavonoid content of the hydro-methanolic and ethyl acetate extracts of *A. tetragonus* plant. This information can be used to understand the chemical composition of the plant and its potential uses in medicine, agriculture, and food safety. Phenolic and flavonoid compounds are natural compounds found in plants and are known for their antioxidant, anti-inflammatory, and anti-cancer properties. The present study demonstrated that the hydro-methanolic extract of *A. tetragonus* had a higher total phenolic content than the ethyl acetate extract, while the ethyl acetate extract had a higher total flavonoid content than the hydro-methanolic extract. Both extracts exhibited significant differences in their total phenolic and flavonoid content, and showed a strong positive correlation with the antioxidant activity of the extracts. The results of the study suggest that *A. tetragonus* extracts can be used as potential sources of natural antioxidants and could have applications in the development of new nutraceuticals and functional foods. The results suggest that quantitative phytochemical analysis can provide valuable information for assessing the potential biological activity and nutritional value of plant extracts and products, and can aid in the development of new plant-based medicines. In summary, the present study provides a foundation for future research on the phytochemical composition and potential applications of *A. tetragonus* plant extracts. Further studies could lead to the discovery of new bioactive compounds with potential therapeutic applications and could also explore the potential use of these extracts in other fields such as agriculture and food safety.

CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

Author contributions: Author Pooja Sharma performed the wet lab and collected and analysed the data and wrote the entire manuscript. Author Nainesh R. Modi helped in designing and proof reading the article.

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