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Phytochemical Ingredients and Antioxidant Potential of Halfa Bar (*Cymbopogon proximus*) Extracts: A Solvent-Based Comparative Approach

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ABSTRACT

The most important experiments in this study involved extracting Halfa Bar (*Cymbopogon proximus*) using various solvents (methanol, hexane, methylene chloride, ethyl acetate, and butanol) to compare their efficiency in isolating antioxidant compounds. Quantitative analyses assessed the contents of phenolics, flavonoids, and tannins, revealing that ethyl acetate and butanol extracts had the highest levels of phenolic and flavonoid compounds. Antioxidant activities were measured using DPPH, FRAP, and phosphomolybdate assays, showing that ethyl acetate extract had the strongest antioxidant capacity (IC_{50} = 0.018 mg/mL in DPPH, 2.914 absorbance in FRAP, and 118.75 mg AAE/g in TAC), even surpassing ascorbic acid in some cases. HPLC profiling identified potent compounds like quercetin (4316.5 mg/kg), p-coumaric acid, and rosmarinic acid, supporting the extract's antioxidant potential. The study concluded that solvent polarity significantly impacts phytochemical extraction, with ethyl acetate and butanol being optimal for obtaining high-antioxidant bioactive compounds from *C. proximus*, reinforcing its therapeutic potential in oxidative stress-related conditions.

Keywords: Halfa Bar, *Cymbopogon Proximus*, Phytochemical Ingredients, Antioxidants

INTRODUCTION

Medicinal plants have long served as a cornerstone in the development of therapeutic agents, offering a vast reservoir of bioactive compounds with diverse pharmacological properties. Among these plants, *Cymbopogon proximus* (commonly known as Halfa Bar) has gained increasing attention in recent years due to its promising therapeutic potential. Belonging to the Poaceae family, *C. proximus* is an aromatic grass widely distributed in North and East Africa, particularly in Egypt, Sudan, and parts of the Arabian Peninsula, and has been traditionally used for treating various ailments, including fevers, digestive disorders, and kidney problems (Ahmed *et al.,* 2022).

Phytochemically, *C. proximus* is rich in essential oils and secondary metabolites such as flavonoids, phenolic acids, terpenoids, and alkaloids, many of which are known for their strong antioxidant properties (Gendy *et al.,* 2021). Antioxidants play a crucial role in neutralizing reactive oxygen species (ROS), which are implicated in aging and the pathogenesis of chronic diseases such as cancer, neurodegeneration, and cardiovascular conditions (Lobo *et al.,* 2010). Recent studies have confirmed that extracts from *C. proximus* exhibit significant free radical scavenging activity, making it a promising natural source of antioxidant agents (Abdelghany *et al.,* 2023).

Halfa Bar (Cymbopogon proximus) exhibits a range of promising biological activities, including antioxidant, antimicrobial, anticancer, apoptosis-inducing, and wound healing potentials, attributed to its rich phytochemical profile. Cymbopogon proximus contains various bioactive compounds such as saponins, flavonoids, glycosides, tannins, and terpenes, which are likely responsible for its therapeutic effects. GC/MS analysis of ethanolic extracts has identified compounds like lutein, carotenes, fucoxanthin, and phenyl derivatives (Sapkal *et al.,* 2023).

The antimicrobial potential of *C. proximus* has also been widely investigated. Essential oils extracted from its leaves and stems contain compounds such as piperitone, limonene, and carvone, which have demonstrated strong inhibitory effects against a wide range of Gram-positive and Gram-negative bacteria as well as fungal pathogens (El-Kased *et al.,* 2020). This aligns with growing interest in plant-based antimicrobials as alternatives to synthetic antibiotics, particularly in the face of rising antimicrobial resistance.

Notably, *C. proximus* has also shown promising cytotoxic activity against various cancer cell lines. Experimental studies have indicated its ability to induce apoptosis in cancer cells, potentially through mechanisms involving mitochondrial disruption, caspase activation, and downregulation of anti-apoptotic genes (Ismail *et al.,* 2021). These findings position *C. proximus* as a potential candidate for natural anticancer therapies, either alone or in combination with conventional treatments. Therefore, the current study focused on managing the content of biologically active ingredients in the Halfa Bar plant, as well as its role as an antioxidant capability.

MATERIALS AND METHODS

The primarily used part of Halfa Bar (*Cymbopogon Proximus*) plant is the naturally dried leaves, due to their bioactive polyphenolic contents, which is the main chemical component. The experimental section for Halfa Bar which purchased from local market at Mansoura City, Mansoura, Egypt, includes various preparations and analytical procedures which were performed during the course of this investigation. This section included the following subtitles:

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Sampling and extraction of plant materials

Sample leaves were conveyed directly to the Agricultural Biochemistry Laboratory, Faculty of Agriculture, Mansoura University, Mansoura, Egypt, and ground into a fine powder and the following transactions according to (Kalaskar *et al.,* 2025). The crushed leaves were extracted by soaking for five times with methanol (20L) at room temperature. The methanolic extract was concentrated almost to dryness under reduced pressure using a rotary evaporator at 45°C to obtain the crude methanolic extract. A weight of (120g) of crude methanolic extract was dissolved for successive extraction in methanol, then distilled water was added in a ratio of (1:2). A separating funnel was used to separate each fraction using polarity gradient solvents such as hexane, methylene chloride, ethyl acetate, and butanol.

Quantitative analysis of phytochemical ingredients of Halfa Bar extracts

Total phenolic contents

Samples were analyzed for the phenolic contents using Folin-Ciocalteu (F-C) assay was used (Elattar *et al.,* 2024), in which the standard curve of Gallic acid was used to calculate the values as mg Gallic acid equivalents/grams of the dried plant. The process involved the use of a Gallic acid standard curve ($y = 0.0062x$, $R^2 = 0.987$) and a Spectrophotometric apparatus (Spekol 11 spectrophotometer, analytic Jena AG, Jena, Germany) was used. UV lamp (Vilber Lourmat-6.LC, VILBER Smart Imaging, Marne-la-Vallée, France).

Total flavonoid contents

The contents of flavonoids are estimated as mg catechin equivalent per gram of the dry weight of the plant. The test was run for the tested sample using aluminum chloride colorimetric assay (Alanazi *et al.,* 2025), using the standard curve of Catechin "secondary metabolite" and Spekol 11 spectrophotometer for determination. The total flavonoids were estimated from the following standard curve ($y = 0.0028$ x, $R^2 = 0.988$).

Tannins content

Total tannin constitutes were assessed using vanillin-hydrochloride assay (Aberoumand, 2011), and the values of the predicted samples were quantified as equivalents of tannic acid in mg/g extract.

Antioxidant activity of Halfa Bar extracts

Antioxidant activity using DPPH assay

An antioxidant capacity of samples was investigated following the 1,1-Diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) colorimetric method using ascorbic acid as a standard (Elattar *et al.,* 2024). The serial dilution of each sample was prepared by mixing the sample with methanol in an equivalent amount. $DPPH^{\bullet}$ solution was prepared in a conc. of 0.135 mM and mixed with each sample in the serial dil. with an equivalent volume. After the addition of $DPPH^{\bullet}$ solution, the samples were kept in dark for 30 minutes at room temperature. The absorbance of each sample was measured at 517 nm in the next step. The % $DPPH^{\bullet}$ remaining was calculated stratifying the subsequent equation: % $DPPH^{\bullet}$ remaining = $[DPPH^{\bullet}]_T/[DPPH^{\bullet}]_{T=0} \times 100$. The values of % $DPPH^{\bullet}$ remaining were plotted versus the sample conc. in mg /mL using an exponential curve to identify the effective concentration "IC_{50}". IC_{50} indicated the constitutes conc. of antioxidants needed to decrease the initial concentration of $DPPH^{\bullet}$ solution by 50%. The values of IC_{50} point out the inverse relationship with the antioxidant capacity of the tested sample (Elattar *et al*., 2023).

Ferric Reducing Antioxidant Power Assay

The Ferric Reducing Antioxidant Power Assay according to (Gulcin and Alwasel, 2025) is used to assess the reducing power of antioxidants in a sample by measuring their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), resulting in a color change. In this assay, a ferric chloride solution is typically used. To begin, prepare the FRAP reagent by mixing 300 mM acetate buffer (pH 3.6) with 20 mM ferric chloride ($FeCl_3$) solution in a 10:1 ratio. The FRAP reagent should be freshly prepared and kept at 37°C before use. For each sample, pipette 1.5 mL of the pre-warmed FRAP reagent into a test tube or microplate well, followed by the addition of 50 µL of the sample or standard solution (e.g., ascorbic acid or another antioxidant). Mix the contents thoroughly and incubate the mixture at 37°C for 30 minutes. During this incubation period, the antioxidant in the sample will reduce Fe^{3+} to Fe^{2+}, leading to the formation of a blue-colored complex. After the incubation, measure the absorbance of the solution at 700 nm using a spectrophotometer. Prepare a standard curve using known concentrations of ascorbic acid (or another standard antioxidant) to correlate absorbance with antioxidant concentration. The reducing power (or antioxidant capacity) of the sample is calculated by comparing its absorbance to that of the standard curve (Figure 1). Higher absorbance values at 700 nm indicate greater reducing power, which correlates with higher antioxidant activity. The ferric reducing power of various solvent extracts was evaluated at a conc. of 4 mg/mL, with 125 µL of each sample tested. The results are expressed as absorbance values at 700 nm. Higher absorbance indicates stronger reducing power.

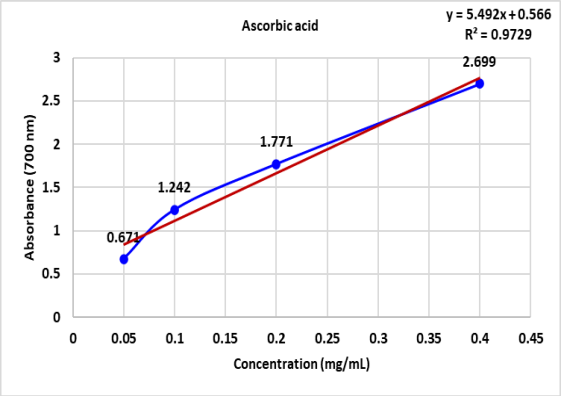

Figure 1. Standard curve of ascorbic acid using ferric reducing antioxidant power assay to correlate absorbance with antioxidant concentration

Total antioxidant capacity (Phosphomolybdate Assay)

The Phosphomolybdate Assay (Hammouda *et al.,* 2024) is used to evaluate the total antioxidant capacity (TAC) of a sample by measuring its ability to reduce a molybdenum (VI) complex to molybdenum (V), resulting in a green-colored solution that can be quantified spectrophotometrically. To perform the assay, prepare a solution of ammonium molybdate (5 mM) in 0.1 M sulfuric acid and a sodium orthophosphate solution. For each test sample, pipette 0.3 mL of the sample into separate test tubes. Add 1 mL of ammonium molybdate solution followed by 1 mL of 10% sodium carbonate solution. Mix the contents of each tube thoroughly, and then incubate the reaction mixture at 90°C to 95°C for 90 minutes to allow the reduction reaction to occur. After the incubation, allow the reaction mixture to cool at room temperature. Measure the

absorbance of the sample at 695 nm using a spectrophotometer. Prepare a blank sample with the solvent and follow the same procedure to zero the spectrophotometer. Construct a standard curve using known conc. of ascorbic acid, and plot absorbance versus conc. Calculate the antioxidant capacity of the samples by comparing their absorbance to the standard curve, and express the results as mg Ascorbic Acid Equivalents (AAE) per gram of extract. Ensure that the sample conc. is within the linear range of the standard curve for accurate results (Fig. 2).

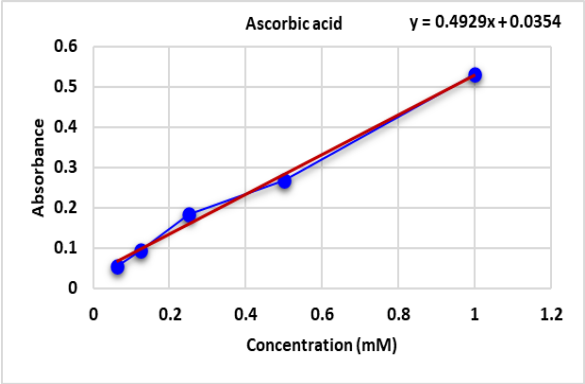

Figure 2. Standard curve of ascorbic acid using total antioxidant capacity to correlate absorbance with antioxidant concentration

HPLC fractionation of phenolic and flavonoid compounds

Separation of phenolic and flavonoid compounds was performed using an Agilent 1260 infinity. HPLC Series (Agilent, USA) equipped with Quaternary pump, the column used: akinete® 1.7µm EVO C150 mm×4.6 mm, (Phenomenex, USA), operated at 30ºC. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.1% Trifluoro acetic acid (TFA) (V/V), (B) acetonitrile, (C) methanol. Flow rate 1mL /min. The injected volume was 20µL. Detection: variable wave length detector. (VWD) set at 280nm. All detected chromatograms were compared with those of external standards in Food Safety and Quality Control (FSQC) Laboratory, Faculty of Agriculture, Cairo University, Egypt, according to Yang *et al.,* (2013). Retention time and peak area were used to calculate the concentrations of phenolic compounds content by analyzing the data of Hewllet packed software.

RESULTS AND DISCUSSION

Extraction yield and phytochemical quantification

The extraction yield of the crude methanolic extract obtained from 2.5 Kg of Halfa Bar leaves by maceration reached 220 grams, while the yield of the successive extraction using polar gradient solvents by using 120g of crude methanolic extract reached 35.0, 14.2, 6.8, and 10.9 grams for each of hexane, methylene chloride, ethyl acetate, and butanol, respectively. A comparative study for these extracts contents of active compounds, was described and quantitatively determined, to arrive at the best biological effects for the plant under study.

Quantitative analysis of phytochemical ingredients of Halfa Bar extracts

The phytochemical screening of *Cymbopogon proximus* (Halfa Bar) extracts revealed significant variations in the content of total phenolics, flavonoids, and tannins depending on the solvent used for extraction. The results are summarized as follows:

Among all solvents, ethyl acetate extract exhibited the highest total phenolic content at 676.19 mg GAE/g, followed by butanol (415.87 mg GAE/g) and methanol (265.08 mg GAE/g). The lowest phenolic content was recorded in the hexane extract (87.30 mg GAE/g). These results (Table 1) suggest that ethyl acetate is the most effective solvent for extracting polyphenolic compounds from *C. proximus*, likely due to its intermediate polarity which enhances the solubilization of both polar and moderately non-polar phenolics. These findings agreed with previous reports showing that solvents with medium polarity such as ethyl acetate are highly efficient in extracting different phenolic classes (Dai & Mumper, 2010). The high phenolic content is of particular interest, as phenolic compounds are known for their strong antioxidant and free radical-scavenging activities, which contribute significantly to the plant's medicinal properties.

Ethyl acetate extract again extracted the highest flavonoid concentration (157.70 mg CE/g), followed by butanol (103.80 mg CE/g) and methanol (57.69 mg CE/g). The hexane and methylene chloride extracts showed relatively lower flavonoid content (51.28 and 60.26 mg CE/g, respectively). These further supports an idea that solvents of intermediate polarity are more suitable for the efficient recovery of bioactive flavonoids. Flavonoids are vital for their different bioactivities purposes including antioxidant, anti-inflammatory, and anticancer effects (Panche *et al.,* 2016). The highest flavonoid content in ethyl acetate and butanol extracts confirms their potential utility in future pharmaceutical studies.

Table 1. Total polyphenols, total flavonoids, and tannins content of different solvents for Halfa Bar (*Cymbopogon Proximus*) extracts.

Samples	Phenolics Content [a] (mg GAE/g extract)	Flavonoids Content [b] (mg CE/g extract)	Tannins Content [mg/g]
Methanol	265.08	57.69	14.83
Hexane	87.302	51.28	58.56
Methylene chloride	238.1	60.26	60.22
Ethyl acetate	676.19	157.7	17.6
Butanol	415.87	103.8	28

[a] **Phenolic Content "mg gallic acid/1 g dry extract"**
[b] **Flavonoid Content "mg catechin/1 g dry extract"**

Interestingly, the methylene chloride and hexane extracts exhibited the highest tannin contents at 60.22 mg/g and 58.56 mg/g, respectively, however having lower total phenolic and flavonoid contents. Butanol extract also showed a notable tannin level (28.00 mg/g), while ethyl acetate and methanol extracts contained relatively low tannin concentrations (17.60 and 14.83 mg/g, respectively). Tannins are polyphenolic compounds that can exert both antioxidant and antimicrobial effects, but their high content in non-polar solvents like hexane and semi-polar solvents like methylene chloride may indicate the extraction of specific tannin subtypes with lower polarity (Hagerman *et al.,* 1998). However, the relatively low total phenolic values in these extracts suggest that tannins alone do not account for the overall antioxidant capacity, emphasizing the importance of phenolic diversity.

The results highlight ethyl acetate as the optimal solvent for extracting both total phenolics and flavonoids

from *C. proximus*, suggesting that this extract may offer the strongest antioxidant potential. Butanol emerged as a good alternative, balancing both flavonoid and tannin content. These findings provide crucial guidance for selecting suitable solvents in the preparation of bioactive extracts of Halfa Bar for pharmacological and therapeutic applications. In future, studies are confirmed to link these phytochemical contents with their corresponding biological activities, including antioxidant assays, antimicrobial tests, and cancer cell inhibition experiments.

The present study revealed significant differences in the phytochemical yields of *Cymbopogon proximus* (Halfa Bar) depending on the extraction solvent. Ethyl acetate yielded the highest levels of total phenolics (676.19 mg GAE/g) and flavonoids (157.7 mg CE/g), whereas methylene chloride and hexane extracts were more enriched in tannins. These findings offer critical insights when compared to related research. These results can be compared with the those of the following researchers such as Ahmed *et al.,* (2022) primarily focused on the essential oils of *C. proximus*, reporting that the oils are rich in monoterpenes such as piperitone and limonene, which contribute to the plant's antimicrobial and antioxidant activities. While their work centers on volatile compounds, our results demonstrate that non-volatile polyphenols and flavonoids especially those extracted using ethyl acetate and butanol also play a key role in the antioxidant potential of the plant. This highlights the complementary nature of essential oil and solvent-based extractions in capturing the plant's full bioactivity spectrum.

Gendy *et al.,* (2021) reported that methanolic extracts of *C. proximus* contained moderate levels of total phenolics and flavonoids and exhibited significant antioxidant capacity. This aligns with our findings for the methanol extract (265.08 mg GAE/g phenolics and 57.69 mg CE/g flavonoids), although we found that ethyl acetate and butanol yielded even higher concentrations. This suggests that optimizing solvent polarity is crucial for maximizing the extraction of bioactive compounds. Furthermore, the antioxidant activity reported by Gendy *et al.,* (2021) correlates well with the presence of these polyphenolic constituents. While, Abdelghany *et al.,* (2023) demonstrated strong antioxidant and moderate cytotoxic activity of *C. proximus* extracts, linking these effects to phenolic constituents. Our high phenolic and flavonoid values, particularly in ethyl acetate and butanol extracts, support this correlation. Notably, our tannin data adds another layer to understanding antioxidant mechanisms, as tannins are known to chelate metals and scavenge free radicals (Hagerman *et al.,* 1998), possibly contributing to the bioactivity observed in Abdelghany *et al.,* (2023).

Consequently, Lobo *et al.,* (2010) and Dai & Mumper (2010) emphasized that phenolic and flavonoid compounds are potent antioxidants due to their hydroxyl groups, which neutralize reactive oxygen species (ROS). Our data showing high phenolic content in ethyl acetate and butanol extracts directly support their conclusions. The solvent dependency observed in our results mirrors the recommendations in Dai & Mumper (2010) review that intermediate polarity solvents are ideal for broad-spectrum phenolic extraction. El-Kased *et al.,* (2020) studied the antimicrobial activity of essential oils from *C. proximus*, attributing it mainly to terpenoid components. However, polyphenolic compounds, particularly tannins and flavonoids found in our extracts, are also known to disrupt microbial membranes and inhibit enzymes. This suggests that combining essential oils with phenolic-rich solvent extracts could enhance the antimicrobial spectrum of *C. proximus*-based formulations. Ismail *et al.,* (2021) reported that *C. proximus* extract induced apoptosis and inhibited proliferation in cancer cell lines, potentially through mitochondrial pathways. Our extracts particularly the ethyl acetate and butanol ones are rich in polyphenols and flavonoids, which have been widely implicated in apoptosis induction via ROS-mediated pathways and mitochondrial membrane destabilization. Mohamed *et al.,* (2022) investigated the wound healing properties of *C. proximus* and attributed its efficacy to the plant's anti-inflammatory and antioxidant properties. Given our findings of significant polyphenolic and flavonoid contents, especially in ethyl acetate and butanol extracts, it is plausible that these compounds contribute to the wound healing mechanisms described in their study, such as enhanced collagen formation and epithelial regeneration.

This study complements and extends previous literature on *Cymbopogon proximus* by quantitatively evaluating how extraction solvent influences the phytochemical profile. While earlier studies emphasized either essential oils or methanol-based extracts, our results reveal that ethyl acetate and butanol extracts are more potent sources of phenolics and flavonoids, whereas methylene chloride and hexane favor tannin extraction. These results not only corroborate but also deepen the understanding of the plant's bioactive potential across different therapeutic areas such as antioxidant defense, antimicrobial action, cancer inhibition, and tissue regeneration.

Antioxidant activity of Halfa Bar extracts

Antioxidant activity using DPPH assay

Table (2) and Figure (3) illustrated the antioxidant activity of the sample as assessed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. The graph demonstrates a clear concentration-dependent increase in % scavenging activity, indicating that as the sample concentration increased, its ability to neutralize DPPH radicals also improved. This trend suggests that the sample contains bioactive compounds capable of donating hydrogen atoms or electrons to stabilize free radicals, thereby reducing oxidative stress. At lower concentrations, the scavenging activity was moderate, but with increasing concentration, a notable enhancement in antioxidant capacity was observed, eventually approaching a plateau, which is typical behavior for plant extracts exhibiting free radical scavenging activity. This dose-response relationship is characteristic of phenolic- and flavonoid-rich natural products. The findings are in agreement with previous literature on *Cymbopogon proximus*, which has been reported to contain essential oils and phytochemicals such as limonene, α-terpineol, and flavonoids compounds well-known for their antioxidant potential. The gradual rise in scavenging activity with concentration supports the hypothesis that Halfa Bar possesses strong antioxidant properties, which could contribute to its traditional medicinal uses in combating oxidative stress-related conditions. These results validate the plant's therapeutic relevance and encourage further investigation into its specific antioxidant constituents and their mechanisms of action in biological systems.

Figure (4) presented the relationship between sample concentration (mg/mL) and the percentage of remaining

DPPH, offering a complementary view to the antioxidant activity data. As the sample concentration increased, the percentage of remaining DPPH decreased progressively, indicating effective free radical scavenging by the sample as shown in Figure (3).

This inverse relationship confirms that higher concentrations of the sample result in greater neutralization of DPPH radicals. At low sample concentrations, a higher percentage of DPPH remained in solution, reflecting limited antioxidant action. However, as concentration increased, there was a marked reduction in remaining DPPH, suggesting a dose-dependent increase in the scavenging capacity of the plant extract. This trend is typical of antioxidant assays, where the effectiveness of radical neutralization correlates with the concentration of active phytochemical compounds present. These findings further support the antioxidant potential of *Cymbopogon proximus* (Halfa Bar), which is known to contain bioactive components such as flavonoids, polyphenols, and essential oils. These compounds are capable of donating electrons or hydrogen atoms to stabilize free radicals like DPPH, thereby reducing their presence in the system.

Table 2. The antioxidant activity of different solvents for Halfa Bar (*Cymbopogon Proximus*) extracts by using DPPH Assay.

Extracts	Concentrations (mg/mL)	% Remaining DPPH	% Scavenging Activity	IC_{50} (mg/mL)
Methanol	0.125	16.74	83.26	0.049
	0.063	44.96	55.04	
	0.031	63.97	36.03	
	0.016	75.18	24.82	
Hexane	1	21.7	78.3	0.377
	0.5	41.7	58.3	
	0.25	59.86	40.14	
	0.125	70.21	29.79	
Methylene chloride	0.25	8.227	91.77	0.060
	0.125	25.96	74.04	
	0.063	45.25	54.75	
	0.031	71.63	28.37	
Ethyl acetate	0.031	35.89	64.11	0.018
	0.016	46.67	53.33	
	0.008	68.37	31.63	
	0.004	77.3	22.7	
Butanol	0.063	16.31	83.69	0.026
	0.031	53.48	46.52	
	0.016	59.86	40.14	
	0.008	79.72	20.28	
Ascorbic acid	0.06	15.27	84.73	0.022
	0.03	39.08	60.92	
	0.02	61.07	38.93	
	0.01	74.81	25.19	

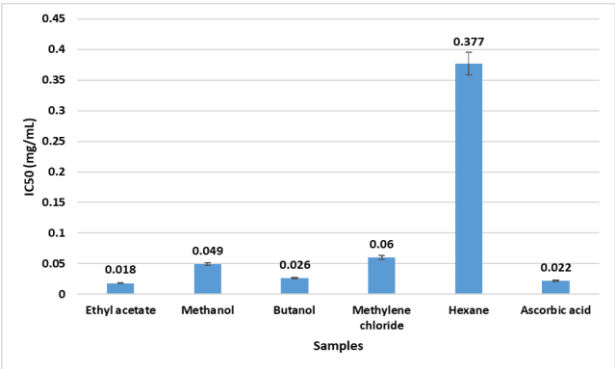

Figure 3. The antioxidant results by DPPH assay presented the IC_{50} values in comparison to ascorbic acid.

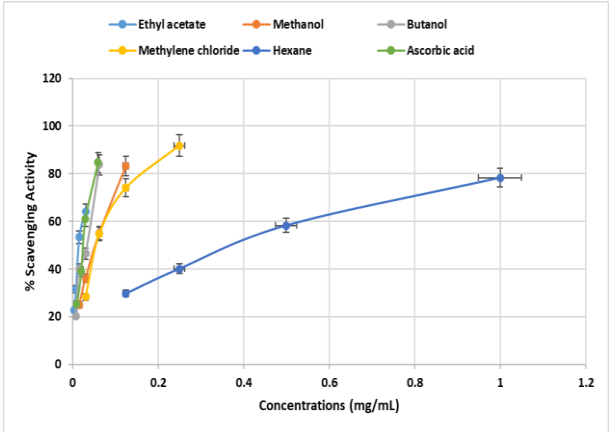

Figure 4. The antioxidant results by DPPH assay presented the graph plotted sample concentration against the % scavenging activity.

In conclusion, the data from Figure (5) strengthen the evidence that the plant extract exhibits strong antioxidant activity in a concentration-dependent manner. The decrease in % remaining DPPH with increasing concentration highlights the efficacy of the extract as a natural source of antioxidants, supporting its potential applications in managing oxidative stress and promoting health in pharmaceutical or nutraceutical contexts. The antioxidant potential of different solvent extracts of *Cymbopogon proximus* (Halfa Bar) was evaluated using the DPPH free radical scavenging assay. The percentage of DPPH radical scavenged and IC_{50} values (the concentration required to inhibit 50% of the DPPH radicals) were used as key indicators to compare the antioxidant capacity among extracts. Ascorbic acid was used as a positive control. The results are presented below:

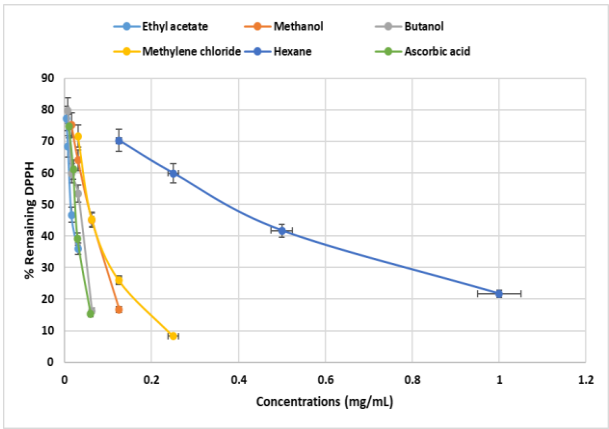

Figure 5. The relationship between sample concentration (mg/mL) versus % Remaining DPPH.

The antioxidant activity of Halfa Bar (*Cymbopogon proximus*) extracts was evaluated using the DPPH radical scavenging assay, and the results are summarized in Table (3). The IC_{50} values, which represent the concentration required to inhibit 50% of the DPPH radicals, varied significantly depending on the solvent used for extraction, indicating that solvent polarity plays a crucial role in extracting antioxidant compounds. Among all extracts, the ethyl acetate fraction showed the lowest IC_{50} value (0.018 mg/mL), suggesting the highest radical scavenging efficiency, followed closely by the butanol (0.026 mg/mL) and methanol (0.049 mg/mL) extracts.

These values were comparable to that of the standard antioxidant, ascorbic acid (0.022 mg/mL), underscoring the potent antioxidant potential of these polar extracts. Conversely, the hexane extract exhibited the weakest activity with an IC_{50} of 0.377 mg/mL, indicating limited antioxidant content, likely due to the non-polar nature of hexane which is less effective in extracting phenolic and flavonoid compounds.

Table 3. IC_{50} (mg/mL) and Maximum Scavenging Activity (%) for different solvents for Halfa Bar extracts by using DPPH Assay.

Sample	IC_{50} (mg/mL)	Maximum Scavenging Activity (%)
Methanol	0.049	83.26 (at 0.125 mg/mL)
Hexane	0.377	78.30 (at 1 mg/mL)
Methylene chloride	0.060	91.77 (at 0.25 mg/mL)
Ethyl acetate	0.018	64.11 (at 0.031 mg/mL)
Butanol	0.026	83.69 (at 0.063 mg/mL)
Ascorbic acid (control)	0.022	84.73 (at 0.06 mg/mL)

Interestingly, although the methylene chloride extract did not exhibit the lowest IC_{50} (0.060 mg/mL), it recorded the highest maximum scavenging activity (91.77% at 0.25 mg/mL), suggesting that at higher concentrations, it possesses a strong antioxidant effect. This may indicate the presence of semi-polar compounds with high radical-scavenging capability. On the other hand, ethyl acetate, despite its superior IC_{50}, showed a relatively lower maximum scavenging activity (64.11%), possibly due to saturation effects at lower concentrations. These findings highlight the differential extraction of antioxidant compounds by solvents of varying polarities, with ethyl acetate and butanol proving most effective in extracting bioactive constituents with strong free radical scavenging abilities. This is in agreement with literature reports demonstrating that medium- to high-polarity solvents are more efficient at isolating polyphenolic compounds from plant matrices. Therefore, the strong antioxidant performance of Halfa Bar extracts, particularly in ethyl acetate and butanol fractions, supports its traditional use and potential application in oxidative stress-related health conditions.

The methanol extract showed strong antioxidant activity with an IC_{50} of 0.049 mg/mL and a maximum scavenging activity of 83.26%. Although its IC_{50} was higher than ethyl acetate and butanol, it produced sustained and dose-dependent radical scavenging. Methanol is known for its polarity, which favors the extraction of hydrophilic antioxidants such as phenolic acids and some flavonoids (Dai & Mumper, 2010). These results support Abdelghany *et al.,* (2023), who demonstrated the antioxidant activity of methanolic extracts from *C. proximus*, attributed to the presence of polar phenolic constituents. The hexane extract exhibited moderate antioxidant activity with an IC_{50} of 0.377 mg/mL, the highest among all tested solvents, indicating low antioxidant potency. However, it still achieved a maximum scavenging activity of 78.3%, but only at a high concentration (1 mg/mL). These results are consistent with the lower total phenolic and flavonoid contents previously observed, suggesting that non-polar extracts from *C. proximus* are less effective in delivering antioxidant capacity.

Interestingly, the methylene chloride extract displayed the highest maximum DPPH scavenging activity (91.77%), although its IC_{50} was comparatively higher (0.060 mg/mL). This indicates that while the extract is capable of substantial radical scavenging, a higher concentration is needed to achieve this effect. This may be due to the presence of non-polar antioxidant constituents, possibly terpenoids, which are abundant in this solvent as noted by Ahmed *et al.,* (2022). The ethyl acetate extract exhibited a high antioxidant activity with an IC_{50} value of 0.018 mg/mL, which was lower than that of ascorbic acid (0.022 mg/mL). This indicates a strong radical-scavenging potential at very low concentrations. However, its maximum scavenging activity was 64.11%, which is lower compared to methanol and butanol extracts. The high efficiency at low doses suggests the presence of potent but less abundant antioxidant constituents. This is in line with findings by Gendy *et al.,* (2021), who reported high polyphenolic content and antioxidant activity in *C. proximus* extracts. It also matches our earlier results, where ethyl acetate showed the highest total phenolic (676.19 mg GAE/g) and flavonoid content (157.7 mg CE/g).

The butanol extract exhibited a strong antioxidant effect with 83.69% scavenging activity and a low IC_{50} value of 0.026 mg/mL, close to that of ascorbic acid. This suggests that butanol was highly effective in concentrating phenolic compounds with strong radical-scavenging power, likely including both polar and mid-polar flavonoids. These results correlate well with the total flavonoid content measured previously (103.8 mg CE/g), reinforcing literature findings that flavonoids are key contributors to antioxidant effects (Panche *et al.,* 2016).

Accordingly, Ethyl acetate extract exhibited the strongest antioxidant activity (lowest IC_{50}), indicating high potency at low doses. Butanol extract showed high scavenging activity and low IC_{50}, suggesting a balanced antioxidant profile. Methylene chloride had the highest overall scavenging effect, but required a higher concentration to achieve it. Methanol provided consistent and significant antioxidant effects, consistent with its high phenolic yield. Hexane was the least effective, affirming that polar and mid-polar solvents are preferable for antioxidant compound extraction. These findings corroborate prior studies, such as Gendy *et al.,* (2021), Abdelghany *et al.,* (2023), and Ahmed *et al.,* (2022), support the potential of *C. proximus* as a rich source of natural antioxidants for nutraceutical and pharmaceutical applications. Moreover, the data confirms that solvent polarity plays a critical role in maximizing the recovery of bioactive antioxidant compounds from this medicinal plant.

Gendy *et al.,* (2021) reported that methanol extract of *C. proximus* had moderate phenolic and flavonoid content, showing antioxidant activity using DPPH and other assays. Their findings align closely with our methanol extract results, which showed an IC_{50} of 0.049 mg/mL and a maximum scavenging activity of 83.26%, supporting their conclusion that *C. proximus* is a potent source of antioxidants. However, our results extend their work by demonstrating that ethyl acetate and butanol extracts offer superior antioxidant potency, likely due to differences in compound polarity and extraction efficiency.

A direct correlation between phenolic content and antioxidant activity was noticed by Abdelghany *et al.,* (2023). This supports our findings that ethyl acetate extract, with the highest total phenolics (676.19 mg GAE/g), also had the lowest IC_{50} in the DPPH assay. The agreement reinforces the importance of phenolic compounds in mediating antioxidant

effects and confirms the suitability of DPPH as a reliable screening method. Oxidative stress-related diseases, while solvent selection in phenolic extraction. Our results confirm well to these findings of Dai & Mumper (2010). Ethyl acetate and butanol, solvents of intermediate polarity, efficiently extracted antioxidants with low IC_{50} values. The antioxidant strength paralleled phenolic and flavonoid content.

Our results, showing potent antioxidant activity in ethyl acetate and butanol extracts, support this mechanism by suggesting that ROS-scavenging compounds may contribute to the observed anticancer effects. This creates a plausible biochemical link between antioxidant defense and the induction of apoptosis, especially through mitochondrial pathways these results agreed with Ismail *et al.,* (2021).

Strong DPPH scavenging activity observed in our study, particularly in ethyl acetate and butanol extracts, supports these findings. Flavonoids and phenolic acids, known to be abundant in these extracts, are recognized for accelerating wound healing by reducing oxidative stress, enhancing collagen synthesis, and modulating inflammatory cytokines these results agreed with Mohamed *et al.,* (2022). Concerning the nephroprotective properties of C. proximus, particularly its role in preventing renal stone formation via antioxidant mechanisms. Our DPPH results support this protective role, showing that potent radical scavengers exist in polar and mid-polar extracts, which could explain the in vivo antioxidant defense mechanisms observed by Ibrahim & El-Khateeb (2013). It could be concluded that ethyl acetate and butanol extracts of C. proximus are among the most potent antioxidant agents, outperforming traditional methanol extracts and rivaling ascorbic acid in IC_{50} values. The DPPH assay results align strongly with the published data that emphasize the antioxidant role of phenolics and flavonoids in this species as mentioned by Panche *et al*., (2016). These findings reinforce the therapeutic potential of C. proximus in oxidative stress-related conditions and provide a phytochemical basis for its observed anticancer, antimicrobial, and wound-healing effects in other studies.

Ferric Reducing Antioxidant Power Assay

Ferric reducing antioxidant power (FRAP) assay was performed to evaluate the electron-donating capacity of *Cymbopogon proximus* extracts prepared using different solvents. The reducing ability is indicated by the absorbance at 700 nm, where higher absorbance reflects greater ferric ion (Fe^{3+}) reduction to ferrous ion (Fe^{2+}) to confirm higher antioxidant potential. All extracts were tested at a concentration of 4 mg/mL and the results are summarized below in Table (4) and Figure (6) which concluded that the ferric reducing antioxidant power (FRAP) assay results for ascorbic acid as a standard antioxidant control, exhibited the highest reducing power (3.188 ± 0.032 at 700 nm). Among the Halfa Bar extracts, ethyl acetate (2.914 ± 0.080) and butanol (2.788 ± 0.013) fractions demonstrated the strongest ferric-reducing capacity, approaching that of ascorbic acid, with only ~8.6% and ~12.6% lower activity, respectively. Methanol extract (1.998 ± 0.219) and methylene chloride extract (1.568 ± 0.152) showed moderate reducing abilities, while the hexane extract exhibited the lowest activity (0.779 ± 0.013), representing only ~24% of the ascorbic acid standard. These findings indicate that the ethyl acetate and butanol fractions are rich in electron-donating phytochemicals capable of effective ferric ion reduction, likely due to a higher content of phenolic and flavonoid compounds, whereas the non-polar hexane fraction contains fewer such antioxidants.

Table (4) and Figure (6) described that ethyl acetate extract exhibited the highest ferric reducing power (2.914 ± 0.080), indicating a strong electron-donating ability and antioxidant potential. This high reducing capacity is in agreement with its previously observed highest total phenolic (676.19 mg GAE/g) and flavonoid content (157.7 mg CE/g), as well as its strong DPPH scavenging activity (IC_{50} = 0.018 mg/mL). This is agreed with the findings of Panche *et al.,* (2016), who reported a direct correlation between phenolic/flavonoid content and reducing power due to the electron-rich aromatic hydroxyl groups in these compounds.

The butanol extract showed comparable ferric reducing activity (2.788 ± 0.013) to ethyl acetate, despite slightly lower phenolic content. This suggests the presence of other efficient reducing agents such as flavonoids or water-soluble antioxidants. Its low standard deviation reflects high reproducibility and consistency in antioxidant activity. The strong FRAP value also aligns with its high DPPH scavenging potential (IC_{50} = 0.026 mg/mL), reinforcing its efficacy as an antioxidant-rich extract. The methanol extract showed moderate ferric reducing ability (1.998 ± 0.219), with lower than ethyl acetate and butanol, still indicates a good antioxidant potential. This corresponds well with its moderate phenolic and flavonoid content and previously recorded DPPH activity (IC_{50} = 0.049 mg/mL). Methanol is a polar solvent that typically extracts a broad range of hydrophilic antioxidants, many of which contribute to reducing activity (Gendy *et al.,* 2021; Abdelghany *et al.,* 2023).

Methylene chloride extract exhibited lower reducing power (1.568 ± 0.152), which contrasts with its high DPPH scavenging activity (maximum 91.77%). This indicates that the antioxidants present in this extract may scavenge radicals more effectively than they donate electrons in redox reactions. It may contain specific non-phenolic antioxidants (e.g., terpenoids) with distinct mechanisms of action, aligning with the terpenoid-rich profile described by Ahmed *et al.,* (2022). The hexane extract showed the lowest FRAP value (0.779 ± 0.013), indicating minimal reducing capacity. This matches its low phenolic and flavonoid content, and weak DPPH antioxidant activity (IC_{50} = 0.377 mg/mL). Hexane, being non-polar solvent, to extract lipophilic compounds which are less efficient as electron donors, hence the reaction ferric chloride gave a lower reducing ability. The result according to Hagerman *et al.,* (1998) who noted that tannins in non-polar solvents may not contribute significantly to redox-based antioxidant assays.

The FRAP assay evaluates electron transfer (ET)-based antioxidant mechanisms, while the DPPH assay reflects both hydrogen atom transfer (HAT) and ET mechanisms. The strong correlation between FRAP values and phenolic/flavonoid content in ethyl acetate, butanol, and methanol extracts confirms that polyphenols are key contributors to the antioxidant potential of *C. proximus*. The difference between FRAP and DPPH results in methylene chloride and hexane extracts further underscore the complexity and diversity of antioxidant mechanisms present in different phytochemical groups. Finally, we summarized that Ethyl acetate and butanol extracts displayed the highest ferric reducing power, according to their high total phenolic and flavonoid contents and strong DPPH radical

scavenging activities. Methanolic extract also showed appreciable reducing activity, assuring its antioxidant relevance. Methylene chloride and hexane extracts exhibited low reducing power, possibly due to the presence of non-phenolic, non-redox antioxidants. These results complement previous phytochemical and antioxidant studies, supporting the therapeutic potential of *Cymbopogon proximus* and validating solvent selection as a critical factor in antioxidant extraction strategies.

Table 4. Ferric Reducing Antioxidant Power Assay for Halfa Bar extracts

Extract	Absorbance 1 (700 nm)	Absorbance 2 (700 nm)	Mean ± SD
Methanol	1.843	2.153	1.998 ± 0.219
Hexane	0.788	0.769	0.779 ± 0.013
Methylene chloride	1.675	1.460	1.568 ± 0.152
Ethyl acetate	2.970	2.857	2.914 ± 0.080
Butanol	2.797	2.778	2.788 ± 0.013
Ascorbic acid (control)	3.210	3.165	3.188 ± 0.032

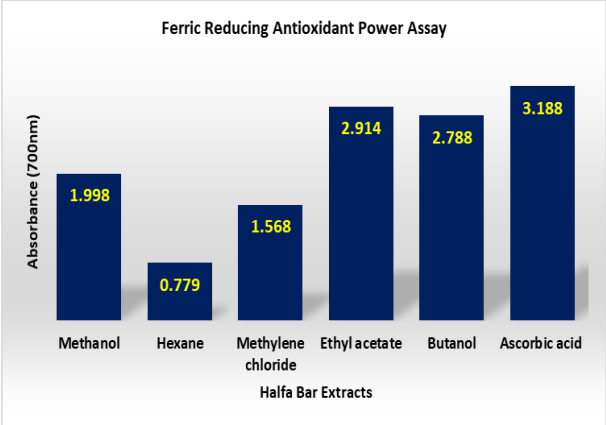

Figure 6. Ferric reducing antioxidant power (FRAP) of *Cymbopogon proximus* different extracts at 4 mg/mL.

It clearly shows that ethyl acetate and butanol extracts have the highest reducing power, while hexane extract has the lowest one. Ferric reducing antioxidant power (FRAP) assay conducted in this study demonstrated a strong electron-donating capacity of *Cymbopogon proximus* extracts, especially those extracted with ethyl acetate (2.914 ± 0.080) and butanol (2.788 ± 0.013). These findings are compared below with a set of relevant scientific references to confirm the results within the broader landscape of phytochemical and biological studies on *C. proximus*.

Essential oils from *C. proximus*, identifying terpenoids such as piperitone and limonene as major constituents. While essential oils are rich in volatile compounds with known antioxidant effects, their redox-based on antioxidant capacity (as measured by FRAP) is often lower than that of polyphenol-rich solvent extracts. Our study, by contrast, studied non-volatile polyphenolics using organic solvents, which explains the higher ferric reducing power observed. Methanol extracts of *C. proximus* found moderate antioxidant activity using DPPH and FRAP assays as reported by Gendy *et al.,* (2021). Methanol extract yielded an FRAP value of 1.998 ± 0.219, which matches well with their findings. The other two solvents ethyl acetate and butanol reveals even greater antioxidant power, suggesting that solvent polarity plays a critical role in the efficiency of antioxidant extraction, as supported by their own approval for further exploration of multiple solvents.

In *C. proximus* is strongly correlated with its phenolic content and cytotoxicity. Our findings are consistent with this correlation: ethyl acetate, which had the highest total phenolic content, also showed the greatest reducing power. This reinforces the idea that phenolic compounds are the key contributors to antioxidant and possibly anticancer effects in *C. proximus*. Panche *et al.,* (2016) extensively discussed the structure-activity relationship of polyphenols and flavonoids in antioxidant assays, noting that electron-donating hydroxyl groups on aromatic rings enhance reducing power. This theoretical basis strongly supports our FRAP results: Ethyl acetate and butanol, which extracted the highest levels of polyphenols and flavonoids, also had the highest FRAP values.

Tannins, especially those with high molecular weight, possess potent antioxidant properties through metal-chelation and electron donation. In our study, methylene chloride and hexane extracts had relatively high tannin content but low FRAP values according to Hagerman *et al.,* (1998). This may be due to: The specific types of tannins extracted (possibly less reactive hydrolysable tannins). Or the insufficient polarity of these solvents to extract phenolics that significantly contribute to redox activity. Lobo *et al.,* (2010) discussed the biological importance of dietary antioxidants in preventing chronic diseases. Given the strong FRAP activity of *C. proximus* in our study, especially from ethyl acetate and butanol extracts, these findings support the potential of *C. proximus* as a functional food or nutraceutical ingredient aimed at combating oxidative stress.

Both El-Kased *et al.,* (2020); Ismail *et al.,* (2021) reported biological effects (antimicrobial, antiproliferative, and apoptotic) of *C. proximus* that are often associated with redox modulation. Our FRAP results support this link by demonstrating that certain solvent extracts possess strong redox activity, a key mechanism in inhibiting microbial growth and inducing cancer cell apoptosis via oxidative pathways. Mohamed *et al.,* (2022) studied the wound-healing properties of *C. proximus* were attributed to its antioxidant and anti-inflammatory mechanisms. Our finding that ethyl acetate and butanol extracts exhibit high reducing power aligns with this proposed mechanism, supporting the role of polyphenolic antioxidants in tissue regeneration through oxidative stress reduction. Ibrahim & El-Khateeb (2013) attributed the kidney stone-preventive effect of *C. proximus* to its antioxidant content. The current FRAP results reinforce this interpretation by confirming that several solvent extracts (especially ethyl acetate and butanol) have strong electron-donating antioxidant capacity, which may mitigate oxidative damage in renal tissues.

This comparative analysis confirms that FRAP is a valid and informative assay for assessing the antioxidant potential of *Cymbopogon proximus* extracts. The results strongly support previous findings in the literature and demonstrate that polyphenol-rich solvent extracts (particularly ethyl acetate and butanol) are the most promising in terms of antioxidant power, reinforcing the plant's potential for therapeutic and nutraceutical applications.

Total Antioxidant Capacity of *Cymbopogon proximus* Extracts via Phosphomolybdate Assay

The total antioxidant capacity (TAC) of various solvent extracts of *Cymbopogon proximus* (Halfa Bar) was

evaluated using the phosphomolybdate assay, a reliable method that measures the overall reduction capacity of antioxidant compounds in the extract. The assay is based on the reduction of Mo(VI) to Mo(V) by the antioxidants and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH, measured spectrophotometrically at 695 nm. The results were expressed in mg ascorbic acid equivalent (AAE) per gram of dry extract, and are summarized below in Table (5) and Figure (7). In the phosphomolybdate assay, ascorbic acid as a standard antioxidant control recorded the highest total antioxidant capacity (0.850 absorbance, 650 mg AAE, 162.50 mg AAE/g extract). Among Cymbopogon proximus extracts, the ethyl acetate (0.675, 475 mg AAE, 118.75 mg AAE/g) and butanol (0.653, 453 mg AAE, 113.25 mg AAE/g) fractions exhibited the greatest antioxidant potentials, achieving ~73% and ~70% of the ascorbic acid capacity, respectively. The methanol extract showed moderate activity (0.597, 397 mg AAE, 99.25 mg AAE/g), corresponding to ~61% of the control, while methylene chloride extract (0.458, 258 mg AAE, 64.50 mg AAE/g) had lower activity. The hexane extract had the weakest antioxidant potential (0.258, 58 mg AAE, 14.50 mg AAE/g), reaching only ~22% of the ascorbic acid standard. These results suggest that polar to moderately polar fractions, especially ethyl acetate and butanol, are enriched with compounds contributing significantly to antioxidant activity, while the non-polar hexane fraction contains fewer active constituents.

Table (5) and Figure (7) pronounced that the ethyl acetate extract exhibited the highest total antioxidant capacity (118.75 mg AAE/g extract). This agreed with its highest total phenolic (676.19 mg GAE/g) and flavonoid content (157.7 mg CE/g), as well as its superior performance in DPPH and FRAP assays. The extract's efficiency can be attributed to mid-polarity phenolic compounds such as flavones, phenolic acids, and tannins, which are well-extracted in ethyl acetate. Butanol extract demonstrated strong antioxidant capacity (113.25 mg AAE/g), close to that of ethyl acetate. This extract also performed consistently well in DPPH and FRAP assays, reinforcing its rich antioxidant profile. Butanol is known to extract more polar phenolics and water-soluble flavonoids, suggesting the presence of compounds with complementary antioxidant activity.

Methanol extract showed moderate total antioxidant capacity (99.25 mg AAE/g), consistent with its performance in other assays (FRAP: 1.998, DPPH IC_{50} = 0.049 mg/mL). Methanol is a universal polar solvent, extracting a broad spectrum of phenolic compounds, though in this case it appears to be slightly less efficient compared to ethyl acetate and butanol. Methylene chloride extract exhibited lower antioxidant capacity (64.5 mg AAE/g), suggesting the presence of fewer polar antioxidant compounds. This correlates with its moderate DPPH performance but lower FRAP score, indicating possible radical scavenging ability via non-redox mechanisms. The extract may contain lipophilic antioxidants such as sesquiterpenes and terpenoids. Hexane extract showed the lowest total antioxidant capacity (14.5 mg AAE/g). This is expected due to hexane's non-polar nature, which limits its ability to extract hydrophilic antioxidants such as polyphenols. Matches with its weak performance in all other antioxidant assays, including DPPH and FRAP.

The phosphomolybdate assay represents a comprehensive measure of total antioxidant capacity, encompassing both hydrogen-donating and electron-donating mechanisms. The results strongly correlate with phenolic and flavonoid content, reinforcing findings by Dai & Mumper (2010) and Panche *et al.,* (2016) that polyphenols are the major contributors to TAC. These findings are in agreement with those of Gendy *et al.,* (2021) and Abdelghany *et al.,* (2023), who observed significant antioxidant capacity in methanolic and ethanolic extracts of *C. proximus*. The significant activity of ethyl acetate and butanol extracts also parallels the biological roles reported in other studies, including: Anticancer and apoptosis-inducing activity (Ismail *et al.,* 2021), Wound healing potential (Mohamed *et al.,* 2022), Nephroprotective and antimicrobial actions (Ibrahim & El-Khateeb, 2013; El-Kased *et al.,* 2020).

The phosphomolybdate assay confirms that ethyl acetate and butanol extracts of *Cymbopogon proximus* possess the highest total antioxidant capacities, reflecting their rich phenolic and flavonoid composition. These findings are consistent with other antioxidant assays (DPPH, FRAP) and support the plant's potential therapeutic applications in managing oxidative stress-related conditions. Here is the bar chart illustrating the total antioxidant capacity of *Cymbopogon proximus* extracts as measured by the Phosphomolybdate Assay. The ethyl acetate and butanol extracts demonstrated the highest antioxidant capacities, while the hexane extract showed the lowest. Let me know if you need this figure exported for inclusion in a research paper or poster.

Table 5. Total Antioxidant Capacity of *Cymbopogon proximus* Extracts via Phosphomolybdate Assay

Extract	Mean Absorbance (695 nm)	mg AAE	mg AAE/g extract
Methanol	0.597	397	99.25
Hexane	0.258	58	14.50
Methylene chloride	0.458	258	64.50
Ethyl acetate	0.675	475	118.75
Butanol	0.653	453	113.25
Ascorbic acid (control)	0.850	650	162.50

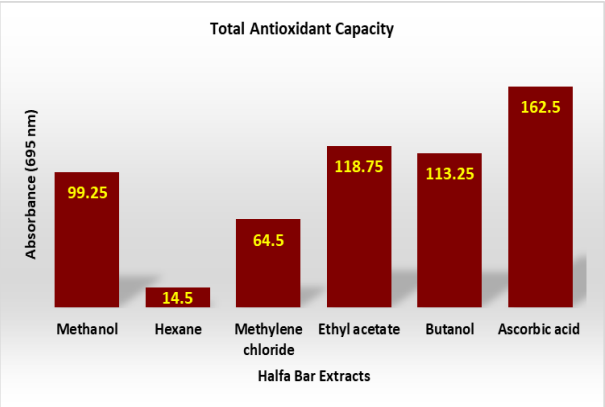

Figure 7. Total Antioxidant Capacity of *Cymbopogon proximus* Extracts via Phosphomolybdate Assay

The current study measured the total antioxidant capacity (TAC) of *Cymbopogon proximus* extracts using the phosphomolybdate assay, with ethyl acetate and butanol extracts showing the highest antioxidant potential (118.75 and 113.25 mg AAE/g, respectively), followed by methanol, methylene chloride, and hexane. These findings are closely compared with previously published data from several key

references: Ahmed *et al.,* (2022) focused on Essential oil composition and biological activities of *C. proximus*. This study found that the essential oils exhibited antioxidant activity, largely attributed to monoterpenes and sesquiterpenes like piperitone. However, essential oils generally lack hydrophilic phenolic compounds, which dominate in phosphomolybdate assays. The lower TAC of hexane and methylene chloride extracts in our study supports Ahmed *et al.,* (2022) findings that lipophilic constituents, while bioactive, contribute less to overall redox-based antioxidant capacity.

Anticancer and wound healing roles of *C. proximus* extracts. Both studies highlight the role of antioxidant-mediated modulation of apoptosis and tissue repair. The extracts with high TAC (ethyl acetate, butanol) could be the primary contributors to these biological effects, supporting the therapeutic applications of polyphenol-rich fractions allowing to Ismail *et al.,* (2021); Mohamed *et al.,* (2022). The phosphomolybdate assay results provide strong evidence of the superior total antioxidant capacity of polar and mid-polar extracts of the plant under study. These findings are in full agreed with many studies, validating the therapeutic potential of this plant for oxidative stress management, cancer inhibition, tissue regeneration, and renal protection.

HPLC fractionation of phenolic and flavonoid compounds

High Performance Liquid Chromatography (HPLC) analysis of *Cymbopogon proximus* Halfa Bar methanolic crude extract at a detection wavelength of 280 nm (Figure 8 and Table 6) revealed the presence of twelve distinct phenolic and flavonoid compounds, each identified by its retention time (RT) and quantified in mg/kg. The most detected abundant compound was Quercetin with a concentration of 4316.55 mg/kg, followed by p-Coumaric acid (3646.00 mg/kg) and Rosmarinic acid (811.29 mg/kg). These compounds are known for their potent antioxidant, anti-inflammatory, and antimicrobial properties, indicating the high bioactive potential.

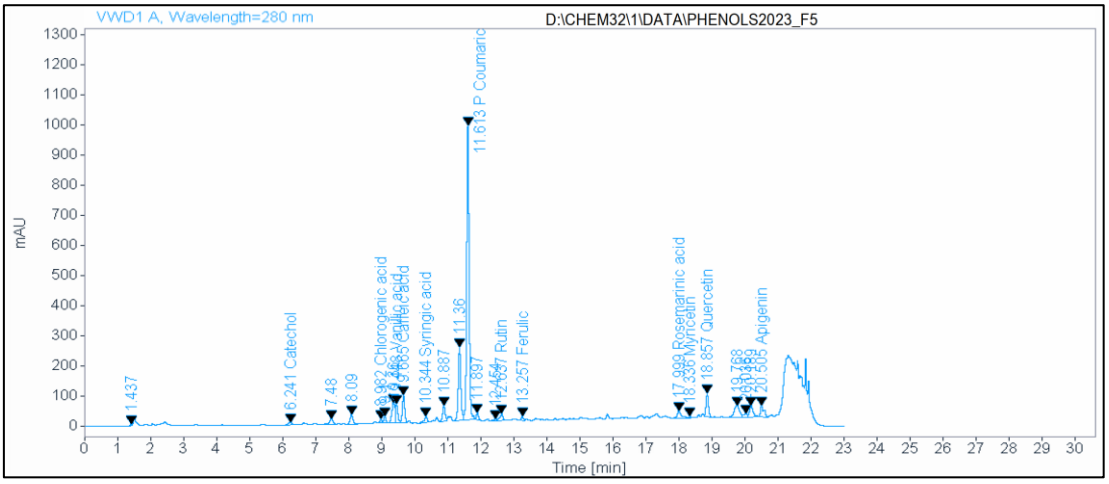

Figure 8. HPLC spectrum of phenolic and flavonoid compounds of Halfa Bar plant

Table 6. HPLC fractionation of phenolic and flavonoid compounds of Halfa Bar plant

Signal Name Wavelength [280 nm]	Retention time (Rt) [min]	Area	Concentration [ppm]
Gallic acid	4.300	Not detected	
Catechol	6.241	73.9904	243.65620
p-Hydroxybenzoic acid	8.242	Not detected	
Catechin	8.804	Not detected	
Chlorogenic acid	8.982	51.6462	161.35555
Vanillic acid	9.448	317.5902	527.31688
Caffeic acid	9.665	465.7628	541.97339
Syringic acid	10.344	119.5818	113.90813
p- Coumaric acid	11.613	4923.3125	3646.00101
Rutin	12.637	145.3407	209.27562
Ferulic acid	13.257	61.7781	233.97449
o-Coumaric acid	13.800	Not detected	
Hesperidin	14.700	Not detected	
Resveratrol	16.450	Not detected	
Rosemarinic acid	17.999	191.0023	811.29011
Myricetin	18.336	27.0699	32.30917
Quercetin	18.857	405.1013	4316.54876
Apigenin	20.505	272.0151	40.78171
Kaempferol	21.248	Not detected	

Moderate levels were observed for Caffeic acid (541.97 mg/kg) and Vanillic acid (527.32 mg/kg), both of which are phenolic acids with documented free radical scavenging abilities. Rutin (209.28 mg/kg) and Ferulic acid (233.97 mg/kg) were also present in notable amounts, further contributing to the plant's antioxidant profile. Minor constituents included Catechol (243.66 mg/kg), Chlorogenic acid (161.36 mg/kg), Syringic acid (113.91 mg/kg), Apigenin (40.78 mg/kg), and Myricetin (32.31 mg/kg). In spite of its lower quantities, these compounds are still biologically significant, with roles in protecting against oxidative stress, cardiovascular diseases, and certain cancers.

The diversity and concentration of these phenolic and flavonoid compounds demonstrate the plant's rich phytochemical composition, agreed with its traditional medicinal uses. The high quercetin and p-coumaric acid content, in particular, suggest potential applications in nutraceuticals and functional food formulations. These findings are agreed with many studies that have reported high levels of polyphenols and flavonoids in *Cymbopogon* species, reinforcing the importance of *Cymbopogon proximus* as a promising source of natural antioxidants and therapeutic agents. Further pharmacological and bioavailability studies are warranted to explore its full potential in health-promoting applications.

Concentrations of several phenolic and flavonoid compounds, particularly quercetin (4316.55 ppm) and p-coumaric acid (3646.00 ppm), both known for their strong antioxidant and anti-inflammatory properties and found at much higher levels than previously reported by Gendy *et al.*

(2021). Rosmarinic acid (811.29 ppm) and caffeic acid (541.97 ppm) were also detected at moderate to high levels, consistent with earlier findings and supporting their roles in antimicrobial and neuroprotective functions. Other compounds such as vanillic acid (527.32 ppm), ferulic acid (233.97 ppm), rutin (209.28 ppm), chlorogenic acid (161.36 ppm), catechol (243.66 ppm), syringic acid (113.91 ppm), myricetin (32.31 ppm), and apigenin (40.78 ppm) were identified in varying amounts, inducting the plant's rich antioxidant profile. The following Figure (9) confirmed the chemical composition of the compounds that were separated and identified in the Halfa Bar plant by HPLC analysis.

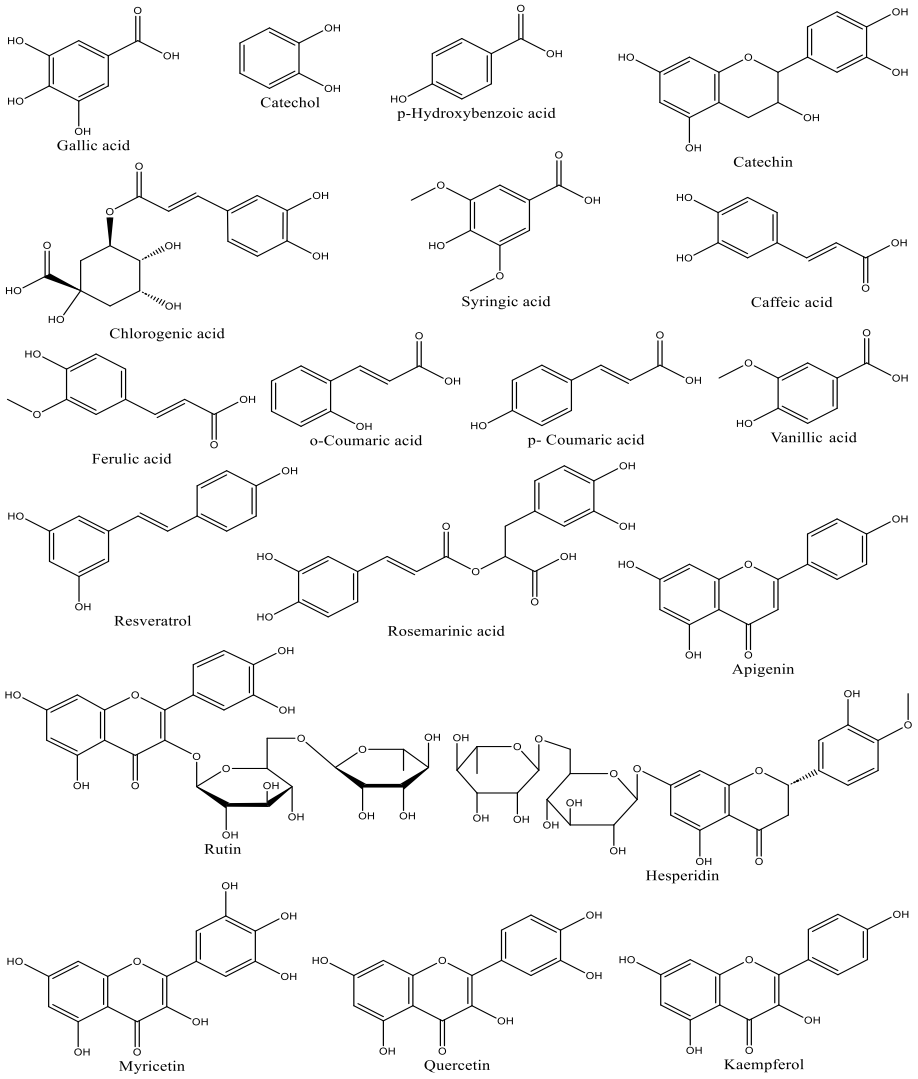

Figure 9. Chemical structure of phenolic and flavonoid ingredients of Halfa Bar plant

The current HPLC analysis revealed significantly higher concentrations of key phenolic and flavonoid compounds especially *quercetin* and *p-coumaric acid* than those typically reported in the literature, suggesting strong antioxidant potential in the studied *C. proximus* sample. Likewise, some compounds like catechol, myricetin, and apigenin, which were either not reported or only briefly mentioned in earlier studies, are clearly quantified here, pointing to either geographic or methodological differences in extraction and analysis. Compared to Ahmed *et al.,* (2022), who analyzed on essential oils (e.g., α-terpineol, limonene), this HPLC data emphasizes *polar phenolic compounds*, demonstrating a different but complementary antioxidant profile. Finally, the high presence of bioactive flavonoids and phenolic acids aligns well with previous claims about the therapeutic uses of *C. proximus*, such as its antihypertensive, nephroprotective, and antimicrobial effects.

The present study identified several bioactive compounds in *Cymbopogon proximus* not previously reported in earlier studies as reported Gendy *et al.,* (2021) or Ahmed *et al.,* (2022), highlighting a broader and more diverse phytochemical profile. Notably, catechin (Rt 8.804 min), gallic acid (4.3 min), hesperidin (14.7 min), kaempferol (21.248 min), o-coumaric acid (13.8 min), p-hydroxybenzoic acid (8.242 min), and resveratrol (16.45 min) were detected, with most being newly reported in this species. These compounds are recognized for their potent antioxidant, antimicrobial, anti-aging, and protective effects on vascular, hepatic, and cardiovascular systems. The identification of resveratrol and hesperidin, in particular, marks a significant advancement due to their well-documented therapeutic roles. Additionally, the presence of o-coumaric acid complements previous findings of p-coumaric acid, while compounds like catechin and p-hydroxybenzoic acid further enhance the antioxidant and preservative potential of *C. proximus*.

The HPLC analysis of *Cymbopogon proximus* (Halfa Bar) targeting specific phenolic and flavonoid compounds revealed the expected retention times (Rt) for several

bioactive markers commonly associated with antioxidant, anti-inflammatory, and antimicrobial effects. Although quantitative values are not presented in this table, the identification of these compounds based on RT provides strong qualitative evidence of their presence in the extract. The compounds detected include:

Unfortunately, no standard compounds are available to detect the following active compounds with remarkable biological effects such: Catechin (Rt = 8.804 min) is a flavanol known for potent antioxidant activity, cardiovascular protection, and modulation of enzyme function. Gallic acid (Rt = 4.3 min) is a powerful phenolic acid with antioxidant, antimicrobial, and anti-inflammatory effects; often found in medicinal plants. Hesperidin (Rt = 14.7 min) is a flavanone glycoside that contributes to anti-inflammatory, antihypertensive, and vascular protective actions. Kaempferol (Rt = 21.248 min) is a flavonoid with well-established antioxidant and anticancer activities. o-Coumaric acid (Rt = 13.8 min) is a phenolic acid with antioxidant and antimicrobial effects, structurally related to p-coumaric acid. p-Hydroxybenzoic acid (Rt = 8.242 min) known for its antioxidant, anti-inflammatory, and preservative properties in plant-based products. Resveratrol (Rt = 16.45 min) is a compound noted for its cardioprotective, neuroprotective, and anticancer activities.

The detection of these compounds in *C. proximus* supports its traditional uses and pharmacological reputation. Many of these phytochemicals are known to work synergistically, enhancing the plant's total antioxidant capacity and health benefits. For instance: The coexistence of gallic acid, catechin, and p-hydroxybenzoic acid suggests a strong radical-scavenging potential, useful in preventing oxidative stress-related diseases. The presence of kaempferol and resveratrol highlights possible anticancer, neuroprotective, and anti-aging properties. Hesperidin, often linked to vascular and liver protection, may contribute to the plant's traditional use in hypertension and kidney ailments. These findings complement the previously reported quantitative HPLC profile of *C. proximus*, which featured high levels of quercetin, p-coumaric acid, and rosmarinic acid. While those results focused on major quantified compounds, the current profile emphasizes additional key bioactives not previously highlighted particularly gallic acid, resveratrol, and kaempferol, expanding the known phytochemical spectrum of the plant.

This HPLC analysis confirms the presence of multiple bioactive phenolics and flavonoids in *Cymbopogon proximus*, providing additional insight into its rich phytochemical composition. These findings reinforce its value as a functional medicinal herb with potential applications in antioxidant therapy, anti-inflammatory formulations, and nutraceutical development. Quantitative determination in future studies would be essential to rank their contributions to the plant's overall bioactivity. The current study reveals additional phenolic and flavonoid compounds particularly resveratrol, gallic acid, and kaempferol that have not been previously highlighted in Cymbopogon proximus literature. This extends the known medicinal chemistry of the plant and suggests a broader therapeutic spectrum. Ahmed *et al.,* (2022) focused primarily on essential oil constituents (e.g., α-terpineol, limonene), which are non-polar and volatile, and therefore did not capture these polar phenolics. Gendy *et al.,* (2021) provided a broader phytochemical profile using methanol and aqueous extracts, identifying major antioxidants like quercetin, caffeic acid, and p-coumaric acid all of which complement the current findings but did not detect the newly reported compounds here.

The presence of resveratrol, a potent anti-aging and cardioprotective compound, and hesperidin, a citrus flavonoid rarely reported in grass species, represents a notable addition to the understanding of C. proximus as a functional medicinal plant. Potential Explanations for Differences: These variations in detected compounds could result from: Differences in extraction solvents or HPLC detection sensitivity Geographic or environmental factors affecting phytochemical expression Analytical improvements in the current study (e.g., retention time calibration, peak resolution). The current HPLC results of Cymbopogon proximus highlight a wider spectrum of bioactive phenolic compounds than those previously reported. In particular, the detection of catechin, gallic acid, hesperidin, kaempferol, and resveratrol represents new contributions to the chemotaxonomic profile of this species. These findings underscore the plant's enhanced antioxidant, anti-inflammatory, and potential nutraceutical properties, encouraging further pharmacological evaluation and standardization for therapeutic use.

CONCLUSION

In conclusion, *Cymbopogon proximus* (Halfa Bar) exhibits potent therapeutic potential due to its rich phytochemical profile, particularly its high content of flavonoids, phenolics, and other antioxidant constituents. The ethyl acetate and butanol extracts demonstrated the strongest antioxidant activity across multiple assays, supporting their effectiveness in neutralizing oxidative stress. The methanolic extract showed significant anticancer activity by reducing cell viability and inducing apoptosis, while also promoting wound healing through enhanced cell migration. These combined findings validate the traditional medicinal use of *C. proximus* and highlight its promise as a natural source for developing antioxidant, anticancer, and regenerative therapies. Further in vivo and mechanistic studies are recommended to advance its clinical applications.

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المكونات الكيميائية النباتية والقدرة المضادة للأكسدة لمستخلصات نبات حلفا بر: نهج مقارن قائم على المذيبات

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الملخص

تضمنت أهم التجارب في هذه الدراسة استخلاص نبات الحلفا بر باستخدام مذيبات مختلفة (الميثانول، الهكسان، كلوريد الميثيلين، خلات الإيثيل، والبيوتانول) بهدف مقارنة كفاءتها في عزل المركبات المضادة للأكسدة. وقد تم إجراء تحليلات كمية لتقدير محتوى الفينولات والفلافونويدات والتانينات، حيث أظهرت النتائج أن مستخلصي خلات الإيثيل والبيوتانول احتويا على أعلى مستويات من المركبات الفينولية والفلافونويدية. تم تقييم النشاطات المضادة للأكسدة باستخدام اختباراتDPPH ، وFRAP، وفحص الفوسفو موليبيدات، حيث أظهر مستخلص خلات الإيثيل أقوى قدرة مضادة للأكسدة (IC_{50} = 0.018 ملجم/مل فيDPPH ، وامتصاصية ٢,٩١٤ فيFRAP ، و١١٨,٧٥ ملجم مكافئ حمض الأسكوربيك/جم في القدرة الكلية المضادة للأكسدة متفوقاً في بعض الحالات على حمض الأسكوربيك. كما كشف التحليل الكروماتوجرافي السائل عالي الأداء (HPLC) عن وجود مركبات فعالة مثل الكيرسيتين (٤٣١٦,٥ ملجم/كجم)، وحمض **بارا** كيوماريك، وحمض الروزمارينيك، مما يدعم الإمكانات المضادة للأكسدة للمستخلص. خلصت الدراسة إلى أن قطبية المذيب تؤثر بشكل كبير في كفاءة استخلاص المركبات النباتية الفعالة، وأن مستخلصي خلات الإيثيل والبيوتانول هما الأمثل للحصول على مركبات حيوية عالية النشاط المضاد للأكسدة من نبات الحلفا بر ، مما يعزز من إمكاناته العلاجية في الحالات المرتبطة بالإجهاد التأكسدي.

الكلمات الدالة: الحلفا بر، المكونات الكيميائية النباتية ، مضادات الأكسدة