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Antioxidant Activity Assessment via 2,2-Diphenyl-1-Picrylhydrazyl and 2,2'-Azino-bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) Assays and Estimation of Total Phenolic and Flavonoid Content in *Portulaca oleracea*

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Abstract

This study investigates the antioxidant therapeutic potential of Portulaca oleracea L. (purslane) harvested from Southern Libya. A comparative analytical approach was employed using three solvents systems of varying polarities (50% Methanol, 50% Ethanol, and Ethyl Acetate) to optimize the recovery of total phenolics (TPC) and flavonoids (TFC). Antioxidant efficacy was rigorously evaluated through 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)and 2,2'-Azino-bis 3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) radical scavenging assays. Results revealed a definitive solvent-dependent hierarchy, with the 50% methanolic extract demonstrating superior extraction efficiency (TPC: 27.25 µg/g; TFC: 5.73 µg/g) and the most potent antioxidant activity. The methanolic fraction recorded the lowest IC_{50} values (58.26 µg /mL for DPPH and 3.1 mg/mL for ABTS), significantly outperforming the ethanolic and ethyl acetate fractions. A robust dose-dependent correlation ($R^2 > 0.9$) was established across all assays, confirming that the high dielectric constant of methanol

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facilitates the maximum recovery of polar bioactive glyco-flavonoids. These findings position Portulaca oleracea as a high-value functional food and a rich source of natural antioxidants for pharmaceutical applications, with 50% methanol being the optimal solvent for maximum phytochemical yield.

Keywords: Portulaca oleracea L, phenolic, flavonoids Free Radicals, Antioxidants, Extract

تقييم النشاط المضاد للأكسدة باستخدام اختبارات 2,2- ثنائي فينيل-1- بيكرايل هيدرازيل و2,2'- أزينو-بيس(3-إيثيل بنزوثيازولين-6-حمض السلفونيك) وتقدير إجمالي محتوى الفينولات والفلافونويدات في نبات الرجلة (Portulaca oleracea).

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الملخص

قامت الدراسة بقياس فعالية نبات الرجلة (*Portulaca oleracea* L.) الذي تم جمعه من المنطقة الجنوبية بدولة ليبيا كمضادات الأكسدة واعتمدت الدراسة نهجاً تحليلياً مقارناً لتقييم تأثير ثلاثة مستخلصات متفاوتة القطبية لكل من المذيبات (50%ميثانول، 50% إيثانول، وأسيتات الإيثيل). وذلك بتطبيق اختبارات تثبيط الجذور الحرة باستخدام كاشفي 2,2-ثنائي فينيل-1-بيكرايل هيدرازيل (DPPH) و2,2'-أزينو-بيس 3-إيثيل بنزوثيازولين-6-حمض السلفونيك (ABTS) . حيث لوحظ ان المستخلص الميثانولي سجل ادني قيمة ل IC_{50} بلغت (58.5 ميكروغرام /مل) لاختبار DPPH و(3.1 ميكروغرام /مل) لاختبار ABTS مقارنة بمستخلصي الإيثانول وأسيتات الإيثيل وبالتالي له اعلى كفاءة كمضاد للاكسدة .اما بالنسبة للمحتوي الكلي للفينولات والفلافونيدات

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لوحظ ان المستخلص الميثانولي سجل اعلى كفاءة استخلاص من المحتوى الفينولي بلغت27.25 ميكروغرام/غرام و المحتوى الفلافونيدي: 5.73 ميكروغرام/غرام) . من خلال الدراسة تم إثبات وجود ارتباط قوي يعتمد على ($R^2 > 0.9$) في جميع الاختبارات، مما يؤكد أن ثابت العزل الكهربائي العالي للميثانول يُسهّل استخلاص أقصى قدر من جليكو–فلافونويدات القطبية النشطة بيولوجيًا. تُصنّف هذه النتائج نبات الرجلة (Portulaca oleracea) كغذاء وظيفي عالي القيمة ومصدر غني بمضادات الأكسدة الطبيعية للتطبيقات الصيدلانية، حيث يُعدّ الميثانول بتركيز 50% المذيب الأمثل للحصول على أعلى إنتاجية من المركبات الكيميائية النباتية.

الكلمات المفتاحية: نبات الرجلة(*Portulaca oleracea* L.) ، المركبات الفينولية، الفلافونويدات، الجذور الحرة، مضادات الأكسدة، الاستخلاص

1. Introduction

Purslane (Portulaca oleracea L.), also known as common purslane, is a widespread succulent plant belonging to the Portulacaceae family. Although native to India and the Middle East, purslane exhibits adaptability to various global climatic conditions (Kumar et al., 2021**)**. While classified as a short-day plant, it demonstrates versatility in different environments (Minh et al., 2019**)**. This plant is rich in bioactive compounds, including flavonoids, alkaloids, omega-3 fatty acids, vitamins, and minerals (Fukalova et al., 2022**)**, which contribute to its medicinal properties such as cooling, fever-reducing, diuretic, antiparasitic, and anti-dysenteric effects. Purslane, recognized by the World Health Organization, is considered one of the most important medicinal herbs (Zhou a et al., 2015**)**. It has been reported that the common purslane plant contains many biologically active compounds (Naeem et al., 2013)(Gharneh et al., 2012)(Stroescu et al., 2013). Purslane is reportedly a rich source of omega-3 fatty acids, particularly alpha-linolenic acid, which is considered beneficial for preventing heart disease (Omara-Alwala et al., 1991)(Simopoulos et al., 1992)(Oliveira et al., 2009). (Naeem et al., 2013) stated that purslane contains calcium and magnesium in a 1:1 ratio. Moreover, purslane is a rich source of vitamin A and other antioxidants such as vitamin C, tocopherol, and beta-carotene (Uddin a et al., 2014). According to published studies, the total phenolic content in purslane varieties ranges from

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127 to 478 mg gallic acid equivalent/100 g fresh weight, and their DPPH free radical scavenging activity ranges from 2.52 to 3.29 mg/ml (Alam et al., 2014)(Lim et al., 2009). Flavonoid concentrations vary in different parts of the plant, with the highest concentration found in the root, followed by the stem and then the leaves. Kaempferol, apigenin, luteolin, myricetin, and quercetin are among the most important flavonoids in purslane (P. oleracea L.). The image of the plant harvested from southern Libya is shown in **figure 1** below.

Figure 1. The leaves and stem of the Portulaca oleracea plant harvested from southern Libya

(D'Andrea et al., 2014)was showed that mature purslane (Portulaca oleracea) plants exhibited higher total phenolic content and greater antioxidant activity compared to plants in their immature stages A study evaluated the antioxidant capacity of purslane extracts and their solvent fractions (water, butanol, chloroform, and normal hexane). Phytochemical analysis revealed a variety of bioactive compounds. The DPPH assay showed IC_{50} values of 23 µg/mL for methanol, 25 µg/mL for water, 28 µg/mL for butanol, and 41 µg/mL for chloroform. The highest total flavonoid content was recorded in the methanol extract (137.72 mg equivalent of quercetin/g), and the highest phenolic compound content was also recorded in the methanol extract (176.70 mg equivalent of gallic acid/g). The methanol, butanol, and water

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extracts exhibited a remarkable ability to chelate ferrous ions and a high tannin content. In addition, all extracts showed a high capacity for removing nitric oxide. These results indicate that purslane (Portulaca oleracea) is a valuable source of antioxidants and has potential in combating oxidative damage (Utpal et al., 2021). Its potential as a natural source of antioxidants has been widely investigated, with research showing its capacity to enhance the stability of food products and improve their health profile (Chen et al., 2022). Moreover, its high nutrient content, particularly in terms of essential fatty acids, positions it as an excellent candidate for enriching meat products, which are typically low in these beneficial compounds (Kartikasari et al., 2023).This research aims to determine the total amount of phenolic and flavonoid compounds in the leaves of purslane (Portulaca oleracea L.) and to evaluate its antioxidant activity using DPPH and ABTS tests.

2. Materials and Methods

2.1 . Materials

Fresh purslane leaves were collected from a farm in southern Libya. Plant samples were dried in an oven at 45-50°C for 12 hours, then ground into a fine powder and stored in plastic bottles.

2.2. Chemicals and Reagents

All analytical-grade chemicals and reagents utilized in this study were procured from Sigma-Aldrich (Merck, Darmstadt, Germany) unless otherwise specified.2,2-diphenyl-1-picrylhydrazyl (DPPH, 98%), potassium persulfate ($K_2S_2O_8$, 99%), and Folin-Ciocalteu reagent were employed for radical scavenging and total phenolic content (TPC) quantification **,**Gallic acid (98%) and ascorbic acid (98%) served as the primary standards for calibration, Organic solvents included methanol (95%), n-propanol (99.9%), ethyl acetate (99.5%), and chloroform (99%) ,Sodium carbonate (Na_2CO_3, 98%) and ultra-pure distilled water were used for the preparation of aqueous solutions and pH stabilization.

2.3. Preparation of Plant Extracts

Three different extracts of purslane leaves were prepared using the procedure described by (Uddin et al., 2012) with some

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modifications as follows: The dried plant material was mixed with 100 ml of petroleum ether and incubated for 24 hours in the dark at room temperature. The mixture was then filtered using filter paper to remove chlorophyll and fatty compounds, resulting in a pure plant material. This material was then placed on clear paper and left to air dry for 30 minutes. Five grams of dried purslane leaves were mixed with 100 ml of different solvents ((50% Methanol, 50% Ethanol, and Ethyl Acetate)). After 24 hours, the mixtures were centrifuged at 3000 rpm for 15 minutes. The supernatant was filtered using Whatman No. 1 filter paper, and the solvent was then evaporated under reduced pressure using a rotary evaporator. The resulting crude extract was stored at 4°C until later use.

2.4. Determination of total phenolic content

The total phenolic content of various extracts from purslane leaves was determined using the Folin-Cioalto method as described by (Turkoglu et al., 2007) as follows: Briefly, 1 ml of the extract was diluted in a volumetric flask with distilled water until the volume reached 46 ml. One milliliter of Folin-Cioalto reagent was added, and the contents of the flask were thoroughly mixed. After three minutes, three milliliters of sodium carbonate (2%) were added, and the mixture was left to stand for two hours, shaking occasionally. The absorbance of each mixture was measured at 760 nm. The total phenolic content was determined by plotting the titration curve for gallic acid standard.

2.5. Determination of total flavonoid content

The total flavonoid content in various extracts of purslane leaves was determined according to the method described by (Turkoglu et al., 2007) as follows: 1 ml of the extract was diluted with 4.3 ml of 80% aqueous ethanol solution containing 0.1 ml of 10% aluminum nitrate and 0.1 ml of 1 M aqueous potassium acetate solution. After 40 minutes at room temperature, the spectral absorbance was measured at 415 nm. The total flavonoid content was determined by plotting a calibration curve using the quercetin standard.

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2.6. Measurement of Antioxidant Activity

2.6.1. Testing the Free Radical Scavenging Efficacy of DPPH (2,2-Diphenyl-1-picrylhydrazyl)

The DPPH assay was performed according to the method described by (Kedare et al., 2011**)** with some modifications. A 0.1 mM DPPH solution was prepared in methanol and stored in the dark. For the test, 1 mL of each extract concentration was mixed with 2 mL of DPPH solution. The reaction mixture was incubated in the dark at room temperature (25°C) for 30 minutes. Absorption was measured at 517 nm using UV-Vis spectroscopy. The free radical scavenging activity was calculated using the following formula:

Inhibition Ratio = $[(A_0 - A) / A_0] \times 100$. Where: The values of A and A_o are shown in Table 1.

Table1: The Abbreviations. The free radical scavenging activity

A_0	represents the absorbance of the control sample (DPPH + methanol)
A	Represents the absorbance of the tested sample.

2.6.2. Free Radical Scavenging Efficacy Test Using ABTS [2,2'-Azinobis(3-ethylbenzothiazolene-6-sulfonic acid)]

The ABTS assay was performed according to the protocol of (Thaipong et al., 2006). ABTS radical cations were generated. ($ABTS^{\bullet+}$) was prepared by mixing a 7 mM ABTS solution with a 2.45 mM potassium persulfate solution and incubating the mixture in the dark for 12–16 hours at room temperature. The $ABTS^{\bullet+}$ solution was then diluted with methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. For analysis, 1 mL of the extract was mixed with 2 mL of the diluted $ABTS^{\bullet+}$ solution. The mixture was incubated for 6 minutes in the dark, and the absorbance was measured at 734 nm. Inhibition ratio = $[(A_0 - A) / A_0] \times 100$.

3. Results and Discussion

3.1. Total phenolics Content (TPC)

The extraction of bioactive phenolics from *Portulaca oleracea* demonstrated a significant dependence on the solvent's dielectric constant and polarity. The extraction efficiency followed a descending hierarchy: 50% Methanol > 50% Ethanol > Ethyl

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Acetate, as detailed in Table 2. These concentrations were quantified relative to the gallic acid standard, which exhibited a mean total phenolic content of 275.14 ±153.89 µg/g in the calibration assay, ensuring a high degree of analytical sensitivity and providing a reliable benchmark for the comparative assessment of the extracts.

Table 2: Total Phenolic Content (TPC) of different extracts.

Extract Type	TPC(µg/g)
Methanolic Extract	**27.25±15.3**
Ethanolic Extract	**23.08± 1.53**
Etylacetate Extract	**0.243±0.15**

As detailed in Table 2, the maximum recovery of phenolic compounds was observed in the 50% methanolic extract (27.25 ±15.3 µg/g), a result that is scientifically justified by the high solubility of low-molecular-weight polyphenols in methanol. The inclusion of 50% water creates a synergistic hydro-alcoholic environment; water acts as a swelling agent that increases the surface area of the plant cell walls, while methanol facilitates the diffusion of both hydrophilic and moderately lipophilic metabolites. In contrast, the 50% ethanolic extract yielded a slightly lower concentration (23.08 ±1.53 µg/g). This can be explained by the lower polarity of ethanol compared to methanol, which limits its ability to extract highly polar phenolic acids. However, the ethanol-water system is highly effective in stabilizing amphiphilic compounds, providing a balanced recovery of flavonoids and phenolic derivatives.The minimal TPC recorded in the ethyl acetate extract (0.243±0.15µg/g) indicates that the majority of phenolic compounds in this *P. oleracea* batch exist in their glycosylated forms (linked to sugar moieties), which are highly polar. Ethyl acetate, as a semi-polar solvent, is selective only towards aglycones (free, non-sugar linked phenolics) and lipophilic fractions, which appear to constitute a very small percentage of the total phenolic profile in this study.The results conclude that hydro-alcoholic mixtures are the most effective systems for a comprehensive phytochemical screening of *P. oleracea*, as they accommodate the wide range of polarities inherent in its secondary metabolites.

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3.2. Total Flavonoid Content (TFC)

The quantification of Total Flavonoid Content (TFC) in *Portulaca oleracea* demonstrated significant variations based on the solvent system employed. The results, standardized against Quercetin Equivalents (QE), are summarized in Table 3. These concentrations were determined using a quercetin calibration curve, where the reference standard exhibited a mean value of 258.49 ± 175.9 µg/g, providing a validated analytical benchmark for the comparative assessment of the flavonoid density across different fractions.

Table 3: Total Flavonoid Content (TFC) of different extracts.

Extract Type	TFC(µg/g)
Methanolic Extract	**5.73±1.76**
Ethanolic Extract	**3.69±1.4**
Etylacetate Extract	**0.07±0.026**

As presented in Table 3, the extraction efficiency followed a descending hierarchy: 50% Methanol > 50% Ethanol > Ethyl Acetate. The 50% methanolic extract yielded the highest flavonoid recovery (5.73 ±1.76 µg/g). This efficiency is scientifically justified by the optimal dielectric constant of the hydro-methanolic mixture, which facilitates the recovery of polar flavonoid glycosides—the primary flavonoid form in *P. oleracea*.This solvent system leverages the high diffusivity of methanol alongside the cell-wall swelling capacity of water to maximize solute mass transfer. Conversely, the 50% ethanolic extract showed moderate recovery (3.69 ±1.4 µg/g), while the ethyl acetate fraction recorded the lowest content (0.07 ±0.026 µg/g). The minimal yield in ethyl acetate is attributed to its semi-polar nature, which is selective towards non-polar aglycones but fails to solubilize the more abundant glycosylated flavonoids present in the plant matrix. In comparison with international literature, these values align with the documented ranges for flavonoid metabolites in halophytic plants. The preference for hydro-alcoholic systems over semi-polar solvents like ethyl acetate confirms that the flavonoids in this *P. oleracea* batch, such as Quercetin and Apigenin derivatives, are predominantly polar in nature. These findings underscore the importance of solvent selection in capturing the comprehensive bioactive profile of the species.

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3.3. DPPH Radical Scavenging Activity of Portulaca Oleracea Extract

The data of three extracts of Portulaca Oleracea was showed in table 4:

Table 4: IC_{50} and Inhibition of different extracts.

Extract Type	IC50 (µg/mL)	Inhibition(%)
50% Methanolic	58.26	91.87
50% Ethanolic	59.45	89.07
Ethyl Acetate	73.50	85.60

As detailed in Table 4, a robust linear correlation was observed between the concentration of the *Portulaca oleracea* methanolic extract and its radical scavenging activity, confirming that its antioxidant potency is strictly concentration-dependent. Antioxidant Activity of the Methanol Extract.

The antioxidant capacity of the Portulaca oleracea methanol extract was evaluated by quantifying its radical inhibition percentage, with ascorbic acid serving as the reference standard. A ten-point calibration curve was established across a concentration range of 40–300 µg/mL. As illustrated in figure 2, a strong linear correlation was observed between the extract concentration and radical scavenging activity, confirming that the antioxidant potency is strictly concentration-dependent.The quantitative indices, particularly the half-maximal inhibitory concentration (IC_{50}) and the maximum inhibition percentage, underscore the efficacy of the methanolic fraction in neutralizing free radicals relative to the standard benchmark. Similarly, Figure 3 illustrates this direct relationship for the methanolic extract; the maximum inhibitory effect reached 91.87% at a concentration of 300 µg/mL, whereas a minimum activity of 39.33% was recorded at 40 µg/mL. Collectively, these findings definitively establish the dose-response nature of the *Portulaca oleracea* methanolic extract's antioxidant performance.

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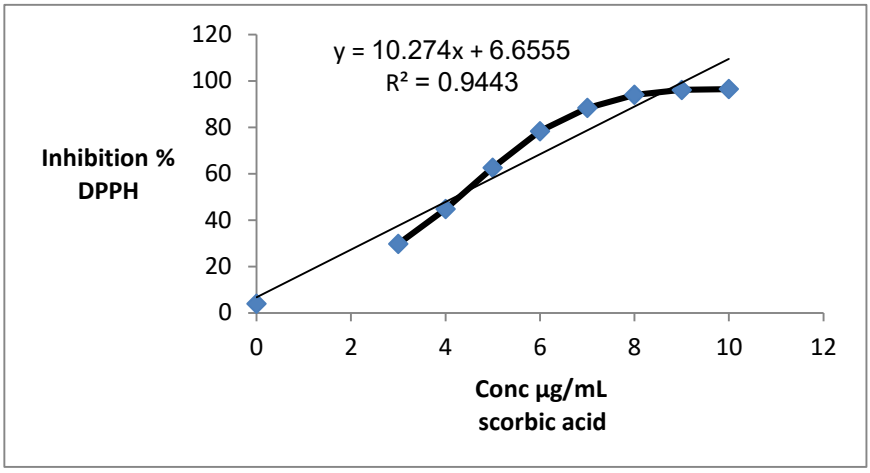

Figure 2: Standard Calibration Curve of Ascorbic Acid for DPPH radical scavenging activity

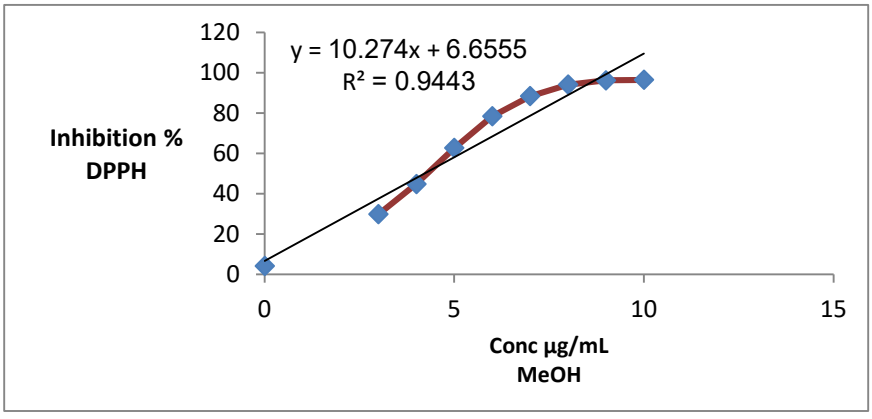

Figure 3: DPPH Radical Scavenging Activity of Methanol Portulaca oleracea Extract

Consistent with the ethanolic profile, figure 4 illustrates a clear dose-response relationship for the ethanolic extract of Portulaca oleracea. The radical scavenging capacity peaked at 89.07% at the maximum tested concentration 300 µg/mL, while the minimum inhibitory activity of 36.53% was recorded at 40 µg/mL. These findings definitively establish the concentration-dependent antioxidant performance of the extract.

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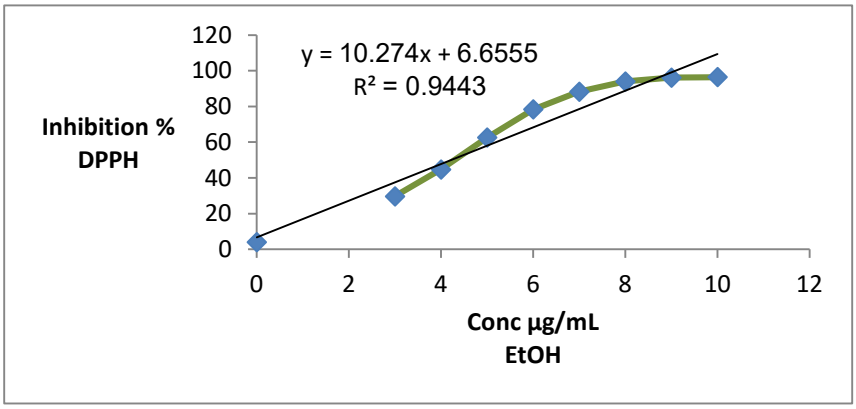

Figure 4: DPPH Radical Scavenging Activity of Ethanol Portulaca oleracea Extract

Mirroring the trends observed in the other solvent fractions, figure **5** demonstrates a robust dose-response relationship for the ethylacetate extract of Portulaca oleracea. The radical scavenging activity reached a peak inhibition of 85.60% at the highest tested concentration of 300 µg/mL, whereas the minimum inhibitory effect of 33.07% was recorded at 40 µg/mL. These results substantiate the concentration-dependent efficacy of the ethanol extract, indicating that the potency of its antioxidant constituents increases proportionally with the extract dose.

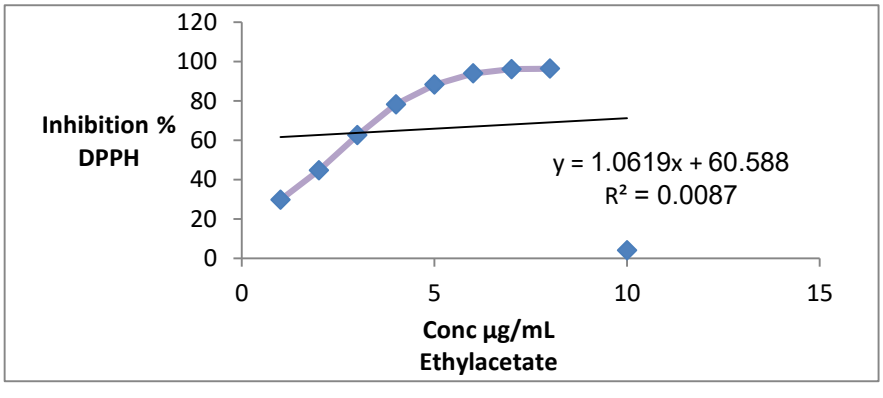

Figure 5: DPPH Radical Scavenging Activity of Ethylacetate Portulaca oleracea Extract

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The experimental data reveals a clear hierarchy in the radical scavenging potency of Portulaca oleracea extracts, ordered as follows: Methanol > Ethanol > Ethyl Acetate. This sequence is directly correlated with the solubility of bioactive compounds in each solvent system. The methanolic extract demonstrated the highest antioxidant efficacy, reaching a maximum inhibition of 91.87% at 300 µg/mL. Methanol's high polarity and low molecular weight facilitate maximum penetration into the plant's cellular matrix. This allows for the comprehensive extraction of a wide spectrum of antioxidant metabolites, particularly polar phenolic acids and glycosylated flavonoids, which possess multiple hydroxyl (-OH) groups capable of high-rate hydrogen atom transfer (HAT). The ethanolic extract followed as the second most effective system, with a peak inhibition of 89.07**%**. The 50% hydro-ethanolic mixture is highly efficient at dissolving amphiphilic antioxidants. While its total recovery is slightly lower than methanol, it maintains a robust dose-response. The minor decrease in activity compared to methanol is attributed to ethanol's slightly lower polarity, which may exclude certain highly polar antioxidant fractions. The ethyl acetate fraction recorded the lowest maximum inhibition at 85.60%. As a semi-polar solvent, ethyl acetate is highly selective, primarily extracting aglycones (non-sugar linked flavonoids).

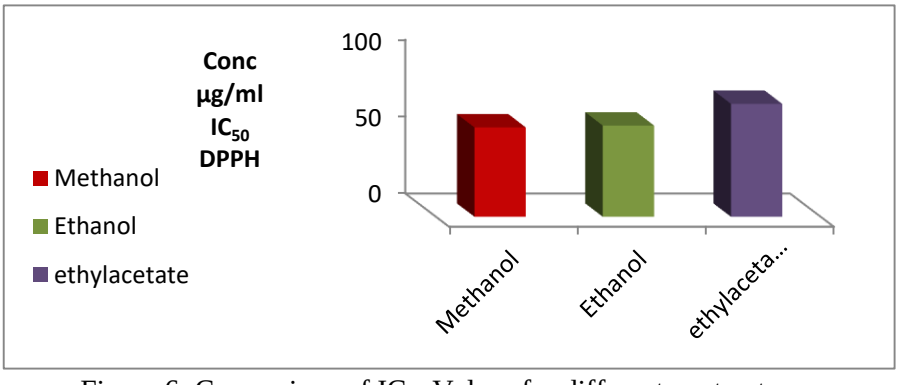

Figure 6: Comparison of IC_{50} Values for different extracts

The IC_{50} value serves as a pivotal metric for evaluating antioxidant efficacy, where a lower value signifies a more potent free radical scavenging capacity. As illustrated in figure 6, the calculated values reveal substantial variations in antioxidant activity among

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the various extracts. The extracts are ranked in descending order of efficacy as follows: Methanol extract (IC_{50} 58.26µg/mL), followed by Ethanol extract (IC_{50} = 59.45µg/mL), and finally Ethyl Acetate extract (IC_{50} = 73.50µg/mL). These results confirm that the methanol extract possesses the superior antioxidant efficacy, characterized by its exceptionally low IC_{50} value, whereas the ethyl acetate extract demonstrated the lowest potency. This observed pattern aligns with the principle that solvent polarity significantly influences the extraction of bioactive compounds. The methanol extract displayed the most robust performance, achieving a maximum DPPH inhibition of 91.87% at a concentration of 0.250 µg /mL. In contrast, the ethyl acetate extract recorded the lowest activity, with maximum inhibition not exceeding 85.60% at 0.221 µg /mL. These findings strongly suggest that the antioxidant properties of Portulaca oleracea are directly dependent on the quantitative and qualitative variation of phenolic and flavonoid compounds recovered using different solvent systems.

3.4. ABTS Radical Scavenging Activity of Portulaca oleracea Extract

The data of three extracts of Portulaca Oleracea was showed in table 5:

Table .5. Data of three extracts of Portulaca Oleracea

Extract Type	IC_{50} (µg/mL)	(%)Inhibition
Methanolic %50	**3.1**	**96.42**
Ethanolic %50	**4.8**	**92.14**
Ethyl Acetate	**45**	**80.00**

As evidenced by the data presented in Table 5, the total antioxidant capacity (TAC) of *Portulaca oleracea* (purslane) extracts was quantified using the ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization assay. This

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spectrophotometric analysis measures the efficacy of the plant's bioactive constituents in neutralizing the blue-green ABTS radical cation (ABTS $^{+}$). The underlying chemical mechanism involves the antioxidant molecules' capacity to donate electrons or hydrogen atoms, thereby reducing the radical into a stable, colorless neutral state. To validate the analytical procedure and establish a comparative baseline, ascorbic acid (Vitamin C) was employed as the reference standard, selected for its superior electron-donating capacity and rapid reaction kinetics. Its high solubility in aqueous media aligns with the polarity of the hydro-alcoholic extracts of *P. oleracea*, providing a validated benchmark for calculating the TAC and ensuring the analytical reliability of the scavenging indices (IC_{50}). A calibration curve was constructed (as shown in Figure 7), exhibiting a robust linear correlation between the concentration of ascorbic acid and its radical scavenging activity. This standard curve functions as a calibrated benchmark, confirming the reliability of the testing parameters. By utilizing this potent reference, the scavenging efficiency of the *Portulaca oleracea* extracts can be precisely quantified and evaluated against a high-performance antioxidant standard.

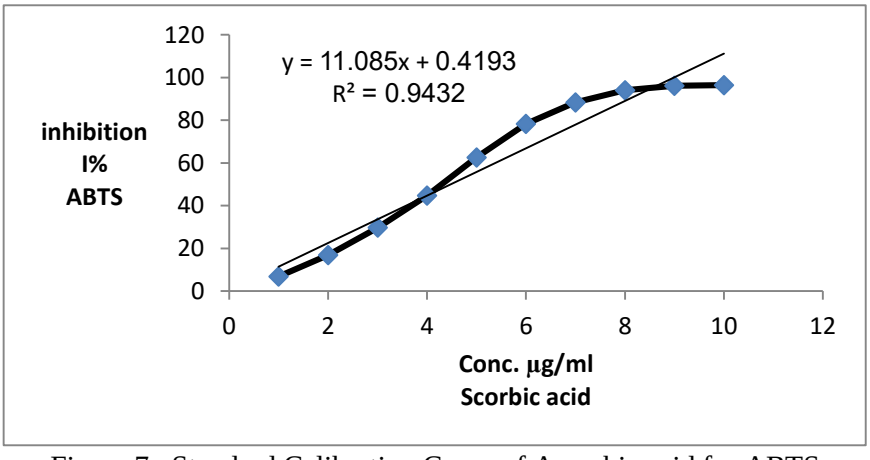

Figure 7: Standard Calibration Curve of Ascorbic acid for ABTS Radical Scavenging Activity

3.4.1. Antioxidant Activity of the Methanol Extract

The antioxidant potential of the *Portulaca oleracea* (purslane) methanolic extract was evaluated using the ABTS radical cation

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scavenging assay. As illustrated in Figure 8, the extract demonstrated a potent inhibitory effect characterized by a distinct dose-dependent response. Quantitative assessment revealed that the maximum scavenging capacity reached 96.42% at the peak concentration of 8.35 µg/mL . Conversely, a baseline inhibition of 5.85% was recorded at the minimum concentration of 0.272 µg/mL. These findings suggest that the methanolic extract possesses a robust capability for neutralizing ABTS + radicals, with its pharmacological efficacy being directly proportional to the concentration of extracted bioactive constituents, was showed that in figure (8).

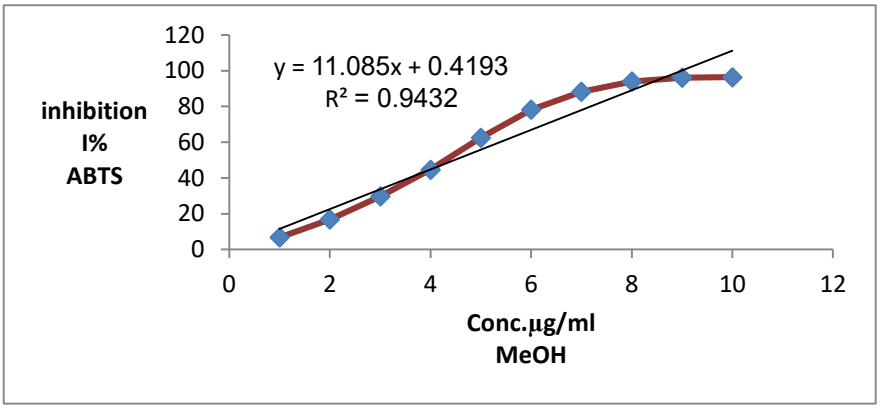

Figure 8: $ABTS^{+}$ Radical Scavenging Activity of Methanol Portulaca oleracea Extract

3.4.2. Antioxidant Activity of the Ethanol Extract

The antioxidant capacity of the *Portulaca oleracea* (purslane) ethanolic extract was quantitatively assessed via the $ABTS^{+}$ radical cation scavenging assay. As illustrated in figure 9, the extract exhibited a potent inhibitory profile characterized by a robust dose-dependent relationship. Analytical data demonstrated that the peak scavenging activity reached 92.14% at the maximum tested concentration of **7.**96 µg/mL. Conversely, a baseline inhibition of 4.80% was recorded at the minimum concentration of 0.91 µg/mL .

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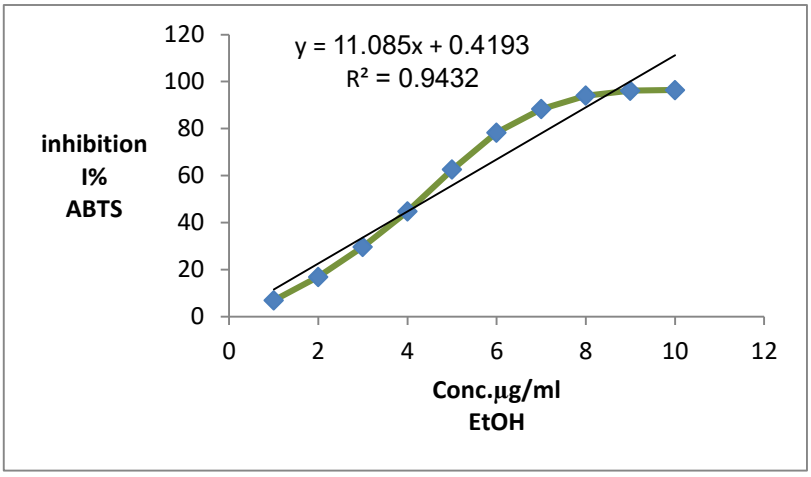

Figure 9: ABTS^{+} Radical Scavenging Activity of Ethanol Portulaca oleracea Extract

3.4.3. Antioxidant Activity of the ethylacetate Extract

The antioxidant activity of the Portulaca oleracea (purslane) ethyl acetate extract was investigated using the ABTS $^{+}$ radical cation scavenging assay. As depicted in figure 10, the extract exhibited a notable inhibitory capacity, adhering to a consistent dose-dependent trend across the evaluated concentrations. Analytical results indicated that the peak scavenging efficiency of 80.00% was attained at the maximum concentration of 6.87 µg/mL Conversely, the minimum inhibitory effect was recorded at 4.00% for the lowest concentration of 0.014 µg/mL . These findings suggest that although the ethyl acetate extract possesses radical-neutralizing properties, its overall potency—particularly at lower concentrations is significantly attenuated compared to more polar extracts. This reduced efficacy is likely attributable to a lower yield of key phenolic and flavonoid constituents within the ethyl acetate fraction .

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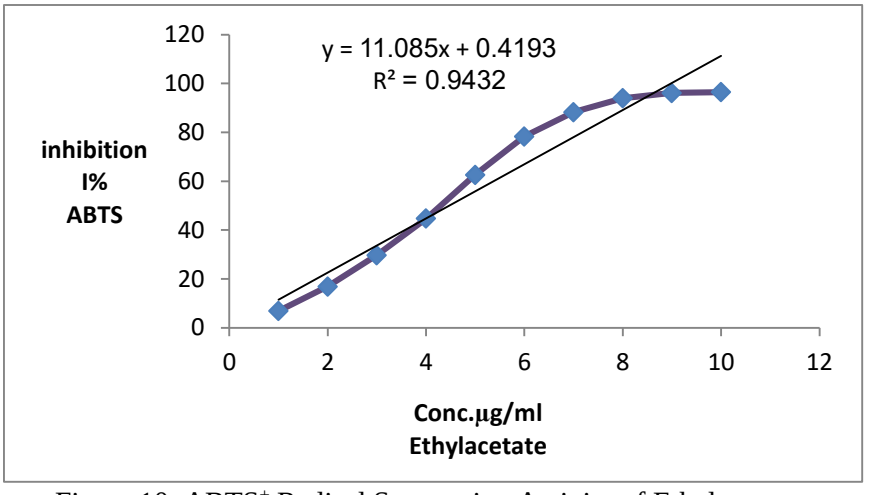

Figure 10: ABTS^{+} Radical Scavenging Activity of Ethylacetate Portulaca oleracea Extract

The extraction solvent's polarity index exerted a profound influence on the antioxidant performance of the Portulaca oleracea extracts. As evidenced by the comparative data in Figure 11, the radical scavenging efficacy followed a definitive hierarchical order: Methanol (IC_{50} = 3.1 µg/mL) > Ethanol (IC50 = 4.8 µg/mL) > Ethyl Acetate (IC_{50} = 45 µg/mL). These results identify the 50% methanolic extract as the most formidable antioxidant fraction, characterized by its remarkably low IC_{50} value, whereas the ethyl acetate extract exhibited the most attenuated activity. This gradient in potency aligns with the fundamental principles of solvent-solute interaction. The high dielectric constant and low molecular weight of methanol facilitate maximum penetration into the plant matrix, ensuring the recovery of high-yield polar bioactive constituents such as hydroxylated phenolics (e.g., caffeic and ferulic acids) and glycosylated flavonoids. These compounds possess multiple hydroxyl (-OH) groups that act as potent electron or hydrogen atom donors, which is the primary mechanism for neutralizing DPPH and ABTS•+ radicals. Furthermore, methanol serves as a protective agent during extraction by inhibiting oxidative enzymes, such as polyphenol oxidase, thereby preserving the chemical integrity and radical-scavenging potency of the metabolites. The superior performance of the hydro-methanolic system may also be

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attributed to the co-extraction of diverse phytochemicals, including betalains and alkaloids. The interaction between these varied classes likely results in a synergistic effect, where the total antioxidant capacity of the complex mixture significantly exceeds the sum of its individual components.In contrast, the significantly higher IC_{50} value recorded for the ethyl acetate fraction (45 µg/mL) suggests that its semi-polar nature is selective only towards a minor portion of non-polar aglycones, failing to solubilize the more abundant and potent polar antioxidants present in Southern Libyan purslane. Consequently, the results definitively establish that a solvent's polarity is the critical determinant for optimizing the recovery of secondary metabolites with high therapeutic potential.

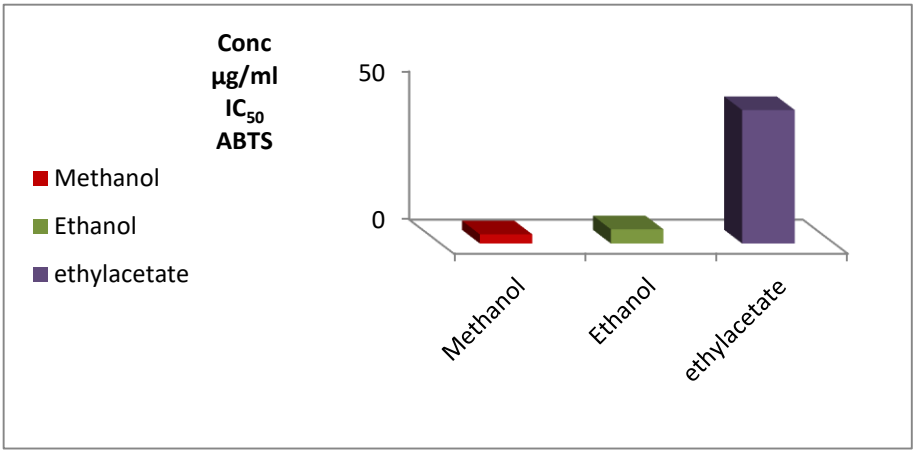

Figure 11: Comparison of IC50 Values for different extracts

4. Conclusion

This study confirms that *Portulaca oleracea* L. harvested from Southern Libya possesses potent antioxidant properties, which are significantly influenced by the extraction solvent's polarity. The comparative analysis reveals that the 50% methanolic extract is the most effective system for recovering both phenolics and flavonoids, leading to the highest radical scavenging activity. In terms of efficacy, the ABTS assay demonstrated a superior sensitivity in detecting the antioxidant potential of the extracts, reaching a peak inhibition of 96.42% with an IC_{50} of 3.1 µg /mL.

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In comparison, the DPPH assay showed a maximum inhibition of 91.87% with an IC_{50} of 58.5 µg /mL. The higher scavenging percentage and lower IC_{50} in the ABTS test suggest that the bioactive compounds in purslane are highly efficient in electron transfer (SET) mechanisms, which are more prominently captured by the ABTS radical cation compared to the DPPH radical. These findings validate the traditional use of *P. oleracea* and highlight its potential as a standardized natural ingredient for the pharmaceutical and nutraceutical industries, particularly in mitigating oxidative stress-related chronic diseases.The future recommendationsTo build upon the current findings, the following technical directions are recommended:

- Phytochemical Characterization: Conduct bio-guided isolation using HPLC-MS to identify the specific molecules (e.g., Quercetin and Apigenin) responsible for the high antioxidant efficacy.
- Green Extraction Optimization: Transition toward 50% Ethanol or Natural Deep Eutectic Solvents (NADES) for industrial applications to meet food-grade safety and "Green Chemistry" standards.
- Bioavailability & Stability Studies: Move from *in-vitro* assays to in-vivo models to assess metabolic pathways, and evaluate the thermal/pH stability of extracts for pharmaceutical formulation.
- Agricultural Standardization: Investigate the impact of Southern Libya's environmental stressors (soil salinity and irrigation) to standardize the phytochemical yield and harvest quality.

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