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Elucidating the Antioxidant Potency and Polyphenolic Profiles of Medicinal Herbs Through Voltammetric and Colorimetric Techniques

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Abstract: This study explores various medicinal herbs' antioxidant capacities and polyphenolic profiles utilizing advanced voltammetric and colorimetric techniques. These herbs, including Rosemary, Cumin, Chamomile, Fennel, Juniper, and Bell Pepper, are renowned for their health benefits, primarily attributed to their high polyphenolic content. We conducted a comprehensive analysis of these herbs' chemical constituents by employing methods such as square wave voltammetry (SWV), Folin-Ciocalteu assay, and ABTS assay. The research demonstrates a strong correlation between the polyphenolic content and antioxidant activity, emphasizing the role of phenolic acids and flavonoids. These findings not only validate the traditional uses of these herbs but also contribute to understanding their potential therapeutic benefits, laying a foundation for future nutritional and pharmaceutical applications.
Keywords: Antioxidant Activity, Polyphenolic Content, Medicinal Herbs, Voltammetry, Colorimetric Assays

1. Introduction

Salvia Rosmarinus, commonly known as Rosemary, is a fragrant evergreen herb indigenous to the Mediterranean region. This herb is versatile, used in cooking for its aromatic qualities, perfumery for its distinctive scent, and as an ornamental plant in gardens. Rosemary is celebrated for its myriad of potential health benefits [1]. Traditionally, it has been used to enhance memory and improve concentration. Additionally, it possesses anti-inflammatory properties that may alleviate muscle pain and soreness. It is also beneficial for digestive health, helping to ease indigestion and stomach cramps [2]. The antimicrobial properties of rosemary also make it effective against bacterial infections [3]. The health benefits of rosemary can largely be attributed to its rich chemical composition, which includes essential oils, phenolic compounds, and flavonoids [4]. Key components such as carnosic acid, rosmarinic acid, 1,8-cineole (eucalyptol), and camphor are instrumental in providing antioxidant, anti-inflammatory, and antimicrobial activities. Rosemary is particularly noted for its high polyphenolic content, making it a potent antioxidant. Polyphenols like rosmarinic and carnosic acid are known for their health-promoting properties [5]. These antioxidants help neutralize harmful free radicals in the body, potentially reducing the risk of chronic diseases such as heart disease and cancer [6]. Furthermore, these antioxidant effects contribute to the anti-aging properties of rosemary, protecting the skin from damage and promoting overall health.

Cuminum Cyminum, commonly known as cumin, is a flowering plant from the family Apiaceae, notable for its seeds that are extensively used in various cuisines worldwide, both in whole and ground form. Cumin is not only valued for its distinctive warm flavor but also for its range of health benefits [7]. Cumin aids in digestion by stimulating the secretion of pancreatic enzymes, necessary for proper digestion and nutrient absorption. It also possesses anti-inflammatory properties that can help alleviate conditions like arthritis and is rich in vitamins such as vitamin C and A, enhancing immune function. Additionally, cumin is a good source of iron, essential for energy production and maintaining immune health. The chemical composition of cumin includes cuminaldehyde, the main component of its volatile oil, which imparts the typical cumin aroma [8]. It also contains beneficial fatty acids like palmitic, oleic, and linoleic acids, alongside proteins and amino acids essential for various bodily functions. Cumin is abundant in polyphenols, antioxidants that protect against oxidative stress and reduce the risk of chronic diseases. The spice contains flavonoids such as apigenin and luteolin and phenolic acids including ferulic, caffeic, and chlorogenic acids. These compounds enhance its antioxidant properties, which combat free radicals in the body—harmful compounds that can cause cellular damage and contribute to chronic diseases like cancer and heart disease. The polyphenolic content and potent antioxidant activity of cumin not only contribute to its health benefits but also make it a valuable addition to a diet focused on preventing oxidative stress and promoting overall well-being [9].

Matricaria Chamomilla, commonly known as Chamomile, is an annual plant from the composite family Asteraceae. Renowned for its aromatic blossoms, chamomile is extensively used in herbal medicine, primarily brewed as a soothing tea. This herb is reputed for its multiple medicinal properties and its benefits encompass a range of therapeutic actions [10]. Chamomile is celebrated for its anti-inflammatory, anti-anxiety, and anti-histamine properties. These make it a versatile herb for treating conditions like skin irritations, reducing stress and anxiety, and managing allergy symptoms [11]. The efficacy of chamomile can be attributed to its complex chemical composition, which includes essential oils like bisabolol—known for antimicrobial and anti-irritant properties—and various flavonoids such as apigenin, which supports its anti-anxiety effects. The plant is also rich in polyphenols such as quercetin and patulin, potent antioxidants that help combat oxidative stress by neutralizing free radicals [12]. These polyphenolic compounds are crucial not only for their antioxidant capacity but also for their role in enhancing immune function, promoting skin health, and preventing inflammation-related disorders [13]. Overall, chamomile's blend of essential oils, flavonoids, and coumarins, along with its high polyphenolic content, contributes significantly to its health-promoting properties. Whether used in teas, extracts, or topical applications, chamomile continues to be a staple in both traditional and contemporary medicinal practices, offering natural remedies for a broad spectrum of health issues.

Foeniculum Vulgare, commonly known as fennel, is a flowering plant belonging to the carrot family. Celebrated for its aromatic and flavorful qualities, fennel is utilized extensively in both culinary and medicinal contexts. The bulbs, foliage, and seeds of fennel are widely employed as seasoning in various cuisines, enhancing dishes with their distinctive flavor [14]. Fennel offers an array of health benefits that include enhancing digestive health by alleviating bloating and improving digestion, and possessing strong anti-inflammatory effects that help reduce bodily inflammation [15]. It is particularly noted for its potent antioxidant properties derived from its rich polyphenolic content. These antioxidants help neutralize harmful free radicals, thus protecting cells from damage and contributing to overall health. The chemical composition of fennel is diverse, including essential oils like anethole, fenchone, and estragole, which are responsible for its unique anise-like aroma. It also contains a variety of phenolic compounds such as rosmarinic acid and chlorogenic acid, known for their antioxidant activities [16]. These compounds, along with various volatile components, contribute to both the herb’s flavor profile and its therapeutic properties. Rich in polyphenols like flavonoids—quercetin and apigenin—and phenolic acids, fennel’s antioxidant properties are robust. These compounds are effective in fighting oxidative stress and inflammation, which are linked to numerous chronic diseases [17]. Additionally, the phytoestrogens in fennel aid in hormonal balance, offering particular benefits to women’s health. Overall, fennel is a multifunctional herb that stands out for its nutritional and health-enhancing properties. It is a valuable addition to the diet, both for its culinary versatility and its numerous health benefits, making it a staple in both kitchens and traditional medicinal practices.

Juniperus Communis, commonly known as juniper, is a species of coniferous tree or shrub belonging to the cypress family. This plant is well-regarded not only for its striking appearance but also for its aromatic berries that are widely utilized as a spice [18]. Juniper berries are especially important in the flavoring of gin and are also a staple in European and Scandinavian cuisines, where they enhance the flavors of various dishes. Juniper berries offer a range of health benefits, from aiding digestion and stimulating appetite to providing antimicrobial and antifungal properties that help combat infections. Additionally, they possess anti-inflammatory properties, which are beneficial in treating conditions such as arthritis and in reducing general inflammation in the body. The chemical composition of juniper is rich and diverse, primarily consisting of essential oils such as alpha-pinene and sabinene, which impart a distinctive aroma and contribute to its therapeutic properties [19]. The berries are also a source of various flavonoids and terpenes like limonene and myrcene, which have been studied for their potential health benefits, including anti-inflammatory and antioxidant effects. Juniper is notably rich in polyphenols, including catechins and proanthocyanidins. These compounds are potent antioxidants, providing significant health advantages such as reducing the risk of chronic diseases, protecting against oxidative stress, and enhancing cardiovascular health. The antioxidant activity inherent in juniper also plays a crucial role in skin health, helping to prevent premature aging and improve overall skin conditions. In summary, juniper is a multifunctional plant that excels both in culinary and medicinal contexts [20]. Its unique flavor-enhancing properties make it a valued addition to food and beverages, while its bioactive compounds offer a range of health benefits, making juniper a cherished ingredient in both kitchens and traditional medicinal practices.

Capsicum Annum, widely known as Bell Pepper or Sweet Pepper, originates from southern North America, northern South America, and Central America. This plant is renowned for producing fruits in an array of vibrant colors such as red, yellow, green, and orange, which are favored in culinary practices for their mild and sweet flavor. Bell peppers are not only celebrated for their culinary versatility but also for their substantial health benefits [21]. They are an excellent source of vitamins C, A, and E—potent antioxidants that support immune function, maintain healthy skin, and prevent cellular damage. Additionally, the high levels of beta-carotene, which the body converts into vitamin A, are crucial for vision and eye health. The peppers also exhibit anti-inflammatory properties, attributed to their rich content of antioxidant vitamins and various phytochemicals, which help mitigate inflammation and reduce the risk of chronic diseases [22]. The chemical composition of bell peppers includes a diverse range of bioactive compounds such as Carotenoids (e.g., beta-carotene, lutein, and zeaxanthin) that enhance eye health and possess antioxidant capabilities, and Flavonoids that contribute to the peppers’ antioxidant activity and vibrant coloration. Significantly, bell peppers are rich in polyphenolic compounds like capsanthin—the primary carotenoid in red bell peppers known for its strong antioxidant properties—and quercetin, which helps reduce inflammation and allergy symptoms. These antioxidants play a critical role in protecting the body from oxidative stress, thereby preventing various chronic conditions [23]. The antioxidant properties of bell peppers not only promote cellular health but also enhance the body’s overall well-being. In summary, Capsicum Annum is valued for more than its flavor; its nutritional profile and health-promoting properties make it an excellent addition to a health-conscious diet. Bell peppers' blend of vitamins, carotenoids, and polyphenols provides powerful protective benefits against health issues linked to oxidative stress and inflammation.

The analysis of total polyphenolic content and antioxidant activity is crucial in fields like food science, pharmacology, and environmental biology, given their roles in health and disease prevention. Commonly utilized methods include voltammetry, Folin-Ciocalteu (FC) assay, and ABTS assay. Each technique offers unique benefits and faces specific challenges. Voltammetry is an electroanalytical technique sensitive to the electroactive properties of polyphenols, making it highly effective for detecting these compounds even at low concentrations [24-27]. This method involves minimal sample preparation and provides real-time data, which is beneficial for rapid screening. However, it requires precise electrode maintenance and calibration, and its performance can vary with different electrode materials and electrolyte compositions. Folin-Ciocalteu Assay is a colorimetric method based on the reduction of a phosphomolybdic-phosphotungstic acid complex by polyphenols under alkaline conditions, resulting in a blue color. It is standardized and widely used for estimating total polyphenol content. Nevertheless, its specificity can be compromised by other reducing substances in the sample. ABTS Assay involves the reduction of a blue-green ABTS radical cation by antioxidants, suitable for assessing both hydrophilic and lipophilic antioxidants. Like the FC assay, it suffers from a lack of specificity as other antioxidants can interfere with the results. Choosing between these methods depends on the study's specific needs, including sample type, available resources, and the desired specificity. Voltammetry is particularly noted for its precision and minimal interference, making it preferable for detailed polyphenolic analysis. However, Folin-Ciocalteu and ABTS assays are more accessible and easier to standardize across different laboratories. Integrating voltammetry with Folin-Ciocalteu and ABTS could harness the strengths of each method, providing a comprehensive analysis of antioxidant capacities and polyphenol profiles in diverse samples. This research focuses on the use of voltammetric electrodes to investigate polyphenolic compounds and develop electrochemical sensors. It compares these methods with colorimetric detection to thoroughly investigate antioxidant activities in complex matrices.

2. Materials and methods

2.1. Chemicals

Gallic acid (97.5-102.5%), a phosphoric acid solution (49-51% HPLC), boric acid (≥99.5% ACS), glacial acetic acid (USP), and sodium hydroxide (≥98% reagent grade) were acquired from Sigma-Aldrich in France. Additionally, further supplies included sodium hydroxide (≥98% reagent grade), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) with a purity of at least 98% according to HPLC, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) at 97% purity, and ammonium persulfate (≥98.0% ACS) from the same supplier. From Merck in the United States, we obtained acetone (≥99.9% HPLC), methanol (≥99.9% HPLC), and ethanol (≥99.8% HPLC). Alumina powder was supplied by Metrohm in France, and ultrapure water from a Millipore (Milli-Q) system was used to prepare the solutions.

2.2. Sample Preparation

Herbal medicines commercially available at local markets in Sohag, Egypt, were obtained for this study. One gram of each herb was ground and homogenized by vigorously shaking in 100 mL of distilled water. The samples were then heated at 50°C for 30 minutes and centrifuged at 5000 rpm for 20 minutes to remove particulates. The final volume of each sample was adjusted to 100 mL with distilled water.

2.3. Solutions

A Britton-Robinson buffer solution is composed of phosphoric acid, boric acid, and acetic acid, each at a concentration of 0.04 mol L^{-1}. To adjust the pH to the required level, ammonium hydroxide (0.2 mol L^{-1}) is utilized. The solutions are shielded from light exposure using aluminum foil and stored at -5 °C in a refrigerator. These stock solutions are prepared on a five-week cycle. The pH measurements throughout the experimental process are conducted using a pH meter from HANNA Instruments.

2.4. Voltammetric Measurements

Voltammetric measurements were carried out using an Autolab PGSTAT128N potentiostat/galvanostat equipped with NOVA 1.10 software, provided by Eco-Chemie in Utrecht, Netherlands. The experiments utilized an electrochemical cell with a 20 mL capacity containing three standard electrodes. The working electrode employed was a glassy carbon electrode (GCE, diameter = 3.0 mm, Metrohm-Autolab, Switzerland), paired with a silver/silver chloride reference electrode (Ag/AgCl, in 3.0 M aqueous KCl) and a platinum wire as the auxiliary electrode. Prior to each measurement, the GCE surface was polished using 0.3 mm alumina powder, thoroughly rinsed with water, and subsequently subjected to ultrasonic cleaning for five minutes. The cleaned electrode was then stored in a Britton-Robinson buffer solution. Cyclic voltammetry was conducted repeatedly until the electrochemical response stabilized. To ensure the reproducibility of the results, three successive measurements were executed.

2.5. Determination of antioxidant activity (AA) by ABTS method

The antioxidant activity (AA) was quantified using the ABTS assay, adapted from the methodology proposed by Guedes et al. [28]. This method involves the preparation of two solutions: one with 7 mmol L^{-1} ABTS and another with 2.45 mmol L^{-1} ammonium persulfate. When mixed, these solutions generate the ABTS radical cation ($ABTS^{\bullet+}$) in an ethanol/water solution (1:1, v/v). The oxidation of ABTS occurs rapidly, yet it requires a stabilization period for the absorbance to reach a steady state. After 24 hours, the $ABTS^{\bullet+}$ solution was diluted with ethanol/water to a target absorbance of 0.700 ± 0.020 at 734 nm.

For the antioxidant assessment of herbal extracts, 1 mL of the diluted $ABTS^{\bullet+}$ solution was combined with 0.1 mL of herbal extract, and the absorbance was measured at 734 nm after a six-minute reaction period. The decrease in absorbance (ΔA =

$A_{ABTS} - A_{extract}$) was calculated for each dilution of the extract. Similarly, absorbance reductions due to the standard antioxidant Trolox were measured across a range of concentrations (50-600 μmol L^{-1}), enabling the creation of a calibration curve for the change in absorbance ($\Delta A = A_{ABTS} - A_{Trolox}$) versus Trolox concentration.

The antioxidant capacity of each extract was then calculated relative to the Trolox calibration curve and expressed as Trolox Equivalents per liter (mol TE L^{-1}) or Trolox Equivalent Antioxidant Capacity (TEAC) values. These measurements were conducted using a JASCO V-750 UV-visible spectrophotometer (JASCO International Co., LTD., Tokyo, Japan) operated with Spectra Manager 2 software.

2.6. Folin-Ciocalteu Comparative Method

The total polyphenolic content of herbal extracts was determined using the Folin-Ciocalteu method, a widely recognized assay utilizing phosphomolybdate and phosphotungstate reagents. In this analysis, gallic acid was used as the reference standard. Measurements were conducted using a JASCO V-750 UV-visible spectrophotometer, with a 1.0 cm glass cuvette providing the optical path for the assay.

3. Results and Discussion:

The square wave voltammetry (SWV) analysis of Salvia rosmarinus extracts reveals three distinct oxidation waves across the examined potential range (0.0 V to 1.6 V vs. Ag/AgCl). These waves are indicative of electroactive species within the extract, likely corresponding to flavonoids and phenolic acids such as carnosic acid and rosmarinic acid. The current (I) measured in microamperes (μA) displays a clear dependence on the concentration of the extract. As observed in Figure 1A, the first oxidation wave appears to initiate at approximately 0.4 V, the second wave peaks near 0.8 V, and the third wave emerges beyond 1.2 V. The increasing peak currents with rising extract concentrations suggest a proportional relationship between the amount of electroactive compounds and the measured current. The oxidation waves demonstrate well-defined peaks, which are characteristic of the oxidation processes of phenolic compounds. The increasing trend in current with potential signifies progressive oxidation. This trend is consistent with the electrochemical behavior of polyphenolic compounds, which typically exhibit multiple oxidation states. The separation of the oxidation waves points to the existence of different compounds within the extract that oxidize at varying potentials. The first wave may be attributed to the compounds that are most easily oxidized, likely phenolic acids, while the subsequent waves may correspond to more oxidation-resistant species, such as flavonoids with multiple hydroxyl groups [29]. From the electrochemical perspective, the appearance of distinct and separated peaks also indicates that the oxidation of these compounds is quasi-reversible, with little overlap between the oxidation processes of different species. This separation of peaks is beneficial for analytical purposes, as it allows for the potential identification and quantification of individual compounds based on their unique oxidation potentials. The systematic shift in peak currents with increasing extract concentration could potentially be used to develop a calibration curve for the quantification of specific compounds within the extract. Figure 1B demonstrates the relationship between the extract concentrations of Salvia Rosmarinus and the SWV peak areas obtained with two standard antioxidant assays, ABTS and Folin-Ciocalteu. The relationship is presented through two distinct linear regressions for each assay, with the current response in microamps (μA) measured at a glassy carbon electrode (GCE) against the extract concentration in parts per million (ppm). The calibration curve for the ABTS assay, marked by blue squares, exhibits a pronounced linear trend with a steeper slope in comparison to the Folin-Ciocalteu assay, which is indicated by red circles. The statistical parameters provided within the figure reveal a higher degree of correlation for the ABTS assay; it boasts a Pearson's correlation coefficient (r) of 0.84792 and a coefficient of determination (R-square) of 0.7203, suggesting a robust linear relationship between the current and the extract concentration. The Folin-Ciocalteu assay, despite having a slightly higher intercept, presents a lower Pearson's r of 0.81533 and an R-square of 0.66476, hinting at a less sensitive response. The square wave voltammetry (SWV) technique is a reliable method for assessing the samples' total polyphenolic content and antioxidant capacity. The SWV method, due to its high sensitivity and low detection limits, allows for the precise quantification of these compounds, which are crucial indicators of the extract's antioxidant properties. The linear correlations with both ABTS and Folin-Ciocalteu assays, as evidenced by the data, reinforce the utility of SWV in the field of antioxidant research, confirming its effectiveness in capturing the electrochemical signatures that directly relate to the polyphenolic content and antioxidant activity within such botanical extracts.

The voltammograms in Figure 2A illustrate the square wave voltammetry (SWV) curves for various concentrations of cumin extract on a glassy carbon electrode (GCE) in a Britton-Robinson (B-R) buffer solution at pH 2. These experiments were conducted with a frequency of 25 Hz, a pulse amplitude of 20 mV, and a pulse width of 5 mV. The observed increase in peak current (i/μA) with increasing concentrations of cumin extract indicates the electroactive nature of components within the extract. Notably, as the concentration increases, the peak currents also increase, suggesting enhanced oxidation of active compounds at the electrode surface. The oxidation peaks, particularly around 0.6 V *vs.* Ag/AgCl, likely correspond to the oxidation of specific flavonoids and phenolic acids present in cumin extract. Flavonoids such as apigenin and luteolin, and phenolic acids like ferulic acid, caffeic acid, and chlorogenic acid, are known for their electroactivity. These compounds can undergo oxidation reactions at distinct potentials, which are observed as peaks in the voltammograms.

The increase in peak currents with the extract concentration suggests these compounds are being oxidized, reflecting their presence and concentration levels in the cumin extract. Figure 2B correlates the total peak area of the square wave voltammograms (TP_{SW}/μA V) with the antioxidant capacity of the cumin extracts, measured by the Folin-Ciocalteu index and the ABTS assay. This figure plots increasing extract concentration against the total polyphenols (expressed in mg GAE L^{-1}) and radical scavenging activity (in mM GA Equivalent, GAE). Both trends exhibit a linear increase in total peak area, indicative of the strong correlation between electrochemical activity and antioxidant properties. The linear relationship between the electrochemical behavior and the Folin-Ciocalteu index supports the concept that the phenolic content, particularly the presence of flavonoids and phenolic acids, largely contributes to the observed electrochemical and antioxidant behaviors. The apigenin, luteolin, ferulic acid, caffeic acid, and chlorogenic acid are significant not only for their electrochemical activity but also for their robust antioxidant properties, which are effectively quantified by these electrochemical techniques. The dual confirmation of electrochemical behavior and antioxidant activity through ABTS assay demonstrates the utility of electrochemical methods as a rapid screening tool for evaluating the antioxidant capacity of herbal extracts. This approach effectively links the electrochemical response directly to the content and efficacy of bioactive compounds, presenting a promising alternative to traditional, more labor-intensive methods used in the evaluation of phytochemicals.

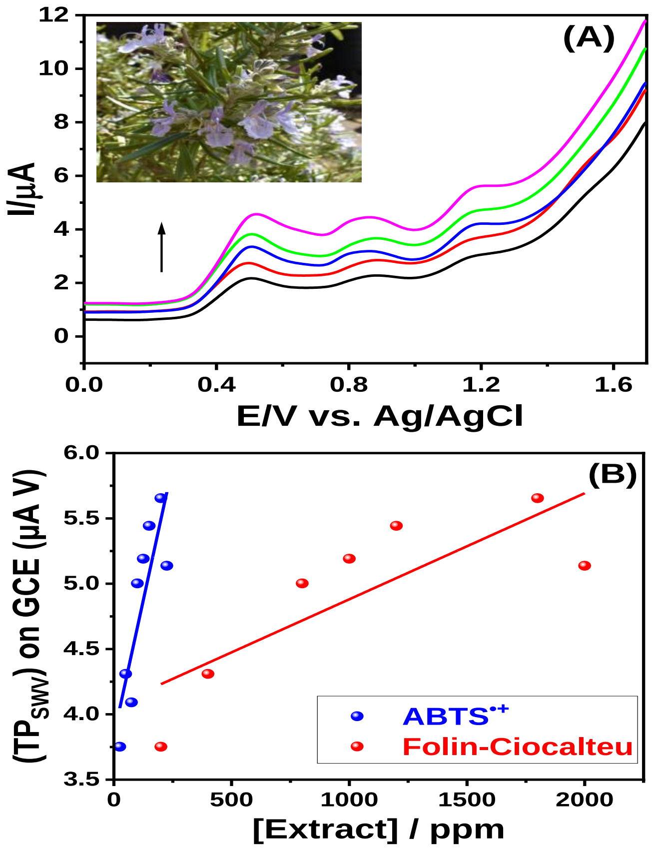

Fig. 1. (A) SWV curves of various additions of Rosemary extract at GCE in B-R buffer solution of pH 2 at a frequency of 25 Hz, pulse amplitude of 20 mV, pulse width of 5 mV. (B) Correlation between total peak area of square wave voltammogram under the voltammogram (TP_{SWV} / µA V) *vs.* Folin Ciocalteu Index (FCI, mg Gallic Acid Equivalent (GAE) L^{-1}) and antioxidant activity determined by the ABTS method (mM GA Equivalent (GAE)).

The provided Figure 3 encompasses two panels that detail the electrochemical analysis and antioxidant properties of fennel extract when interfaced with glassy carbon electrodes (GCE). Figure 3A displays the SWV of various concentrations of fennel extract in a Britton-Robinson (B-R) buffer solution at pH 2, employing a frequency of 25 Hz, pulse amplitude of 20 mV, and pulse width of 5 mV. The voltammograms show progressive increases in peak current with higher concentrations of the extract, evidenced by upward shifts in the curves. Notably, prominent peaks are observable, possibly indicating the presence of flavonoids like apigenin and quercetin, alongside phenolic acids such as rosmarinic acid and chlorogenic acid [30]. These compounds are known for their redox activities, likely responsible for the observed electrochemical behaviors.

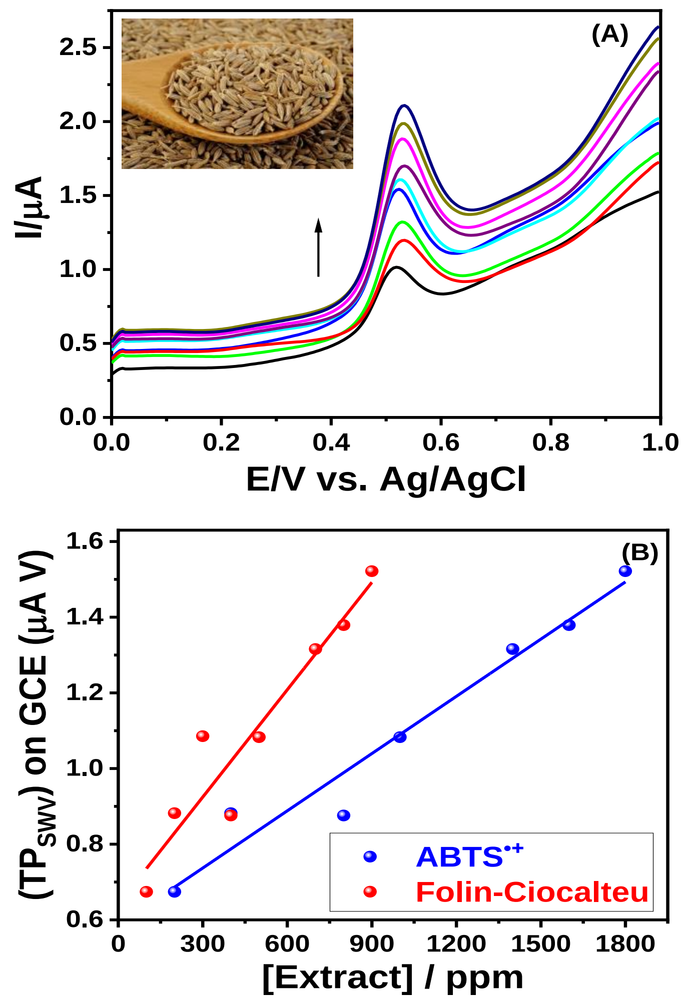

Fig. 2. (A) SWV curves of various additions of Cumin extract at GCE in B-R buffer solution of pH 2 at a frequency of 25 Hz, pulse amplitude of 20 mV, pulse width of 5 mV. (B) Correlation between total peak area of square wave voltammogram under the voltammogram (TPSWV / µA V) vs. Folin Ciocalteu Index (FCI, mg Gallic Acid Equivalent (GAE) L–1) and antioxidant activity determined by the ABTS method (mM GA Equivalent (GAE)).

Figure 3B correlates the total peak area under the voltammograms (TP_{SWV}) with the antioxidant activities measured by the Folin-Ciocalteu and ABTS assays. The analysis reveals a positive linear relationship between electrochemical activity and antioxidant capacities expressed in GAE L^{-1} (mg gallic acid equivalent per liter). Notably, the correlation is more pronounced with the Folin-Ciocalteu method (blue dots and line) compared to the ABTS method (red dots and line). This difference suggests that the Folin-Ciocalteu assay, detecting a broader range of phenolic content, aligns more closely with the electrochemical data. The findings suggest that the peaks in the

SWV are attributable to the redox properties of specific phenolic and flavonoid compounds in the fennel extract. Their ability to undergo oxidation-reduction easily translates to significant antioxidant capabilities, as phenolic acids and flavonoids can donate electrons or hydrogen atoms. The robust correlation between the electrochemical data and the Folin-Ciocalteu assay's results underlines the potential of voltammetric techniques to act as rapid, efficient screening tools for evaluating the antioxidant capacity and phytochemical richness of botanical extracts.

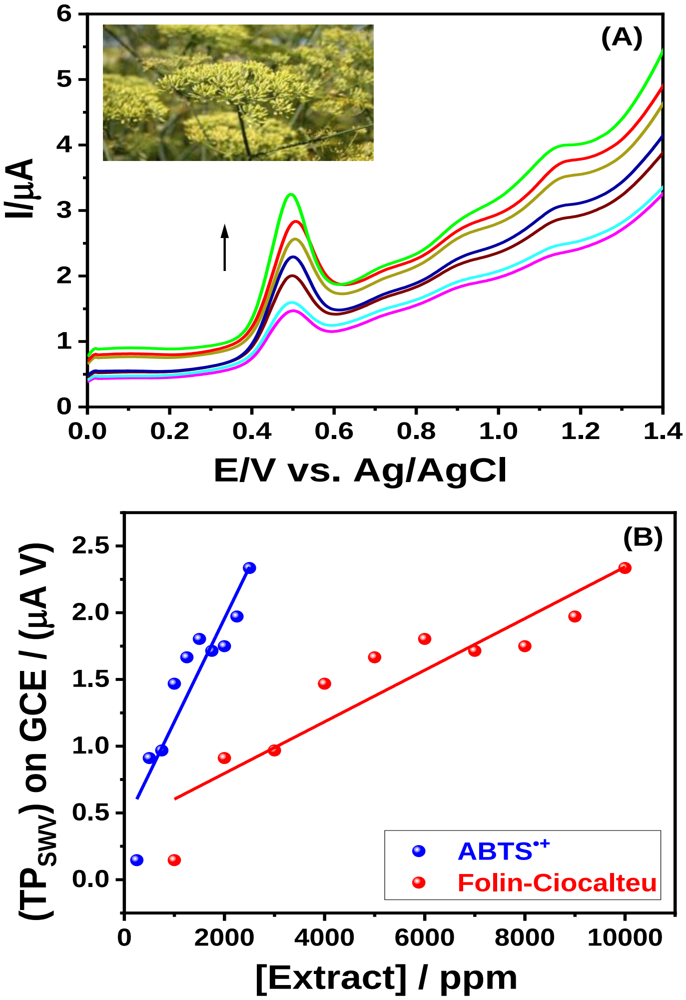

Fig. 3. (A) SWV curves of various additions of Fennel extract at GCE in B-R buffer solution of pH 2 at a frequency of 25 Hz, pulse amplitude of 20 mV, pulse width of 5 mV. (B) Correlation between total peak area of square wave voltammogram under the voltammogram (TP_{SWV} / μA V) *vs.* Folin Ciocalteu Index (FCI, mg Gallic Acid Equivalent (GAE) L^{-1}) and antioxidant activity determined by the ABTS method (mM GA Equivalent (GAE)).

Figure 4 shows both the electrochemical behaviors of juniper extract and its antioxidant properties, through square wave voltammetry (SWV) and correlation with chemical antioxidant assays. Figure 4A depicts the SWV of juniper extract at different concentrations, performed in a Britton-Robinson (B-R) buffer solution at pH 2 with a frequency of 25 Hz, pulse amplitude of 20 mV, and pulse width of 5 mV. The voltammograms exhibit increasing peak currents with increasing extract concentrations, as shown by the progressively elevated curves from the baseline. The sharp peaks, especially highlighted by the arrow, suggest the presence of significant electrochemical activity attributable to specific phenolic compounds, such as catechins and proanthocyanidins, which are known to participate in redox reactions. The SWV data clearly indicates that juniper extract contains electroactive components, catechins and proanthocyanidins, which exhibit oxidation peaks within the applied voltage range. These compounds are phenolics with strong antioxidant capabilities, often contributing to the therapeutic properties of plants. Their electrochemical activity is essential as it reflects their ability to donate electrons, a key mechanism underlying their antioxidant function. Figure 4B correlates the total peak area under the voltammograms with the antioxidant activity measured via the Folin-Ciocalteu and ABTS methods. There is a positive correlation depicted where both antioxidant activity assays increase linearly with the concentration of juniper extract. Notably, the Folin-Ciocalteu method (blue dots and line) demonstrates a steeper slope than the ABTS method (red dots and line), suggesting a more sensitive detection of the phenolic content by the Folin-Ciocalteu assay. The results illustrate that the antioxidant activities measured by both assays are well-correlated with the electrochemical data, affirming the potential of using electrochemical techniques for quick screening of antioxidant properties in botanical extracts.

Figure 5A displays the square wave voltammograms (SWVs) of various concentrations of bell pepper extract measured at a glassy carbon electrode (GCE) in a B-R buffer solution of pH 2. The voltammograms are presented over a potential range from 0 to 1.4 V versus Ag/AgCl. The voltammetric responses increase in current (μA) with increasing extract concentration, indicating that the concentration of electroactive components within the extract correlates positively with the observed current. The increasing peak currents can be attributed primarily to the presence of flavonoids such as apigenin and quercetin in the bell pepper extracts. These compounds are known for their strong antioxidative properties and their ability to undergo reversible redox reactions, which make them readily detectable by electrochemical methods. The oxidation peaks observed in the voltammograms likely correspond to the oxidation of such flavonoids. The slight shifts in peak potential with varying concentration could be a result of changes in the surface activity of the electrode or the interaction between different electroactive species at higher concentrations. In Figure 5B, the correlation between the total peak area of the square wave voltammograms and the antioxidant capacity measured by two different assays—Folin-Ciocalteu and ABTS—is presented. The plot shows a linear increase in TP_{SWV} with increasing concentrations of bell pepper extract (from 0 to 10000 ppm), suggesting a strong relationship between the quantity of extract analyzed and its electrochemical activity. The Folin-Ciocalteu method, indicative of total phenolic content, shows a similar trend to that observed with the ABTS assay, which measures the ability of antioxidants in the extract to scavenge ABTS radical cations. The linear fits provided in the plot (blue for Folin-Ciocalteu and red for ABTS) exhibit high coefficients of determination (R^2 values close to 1), implying that both phenolic content and radical scavenging activity contribute to the electrochemical signals detected. This result supports the hypothesis that higher concentrations of polyphenolic compounds, which include flavonoids like apigenin and quercetin, enhance the antioxidant capacity of the extracts, thereby increasing the voltammetric response.

The presented data elucidate the potential of using electrochemical techniques to quantify the antioxidant properties of natural extracts based on their phenolic content. The correlation between voltammetric signals and standard chemical assays for antioxidant activity confirms the viability of electroanalytical methods for rapid and sensitive analysis of phytochemicals in food and plant extracts. Future studies could expand on these findings by exploring other electroactive compounds within bell pepper extracts and their specific contributions to the voltammetric signals observed.

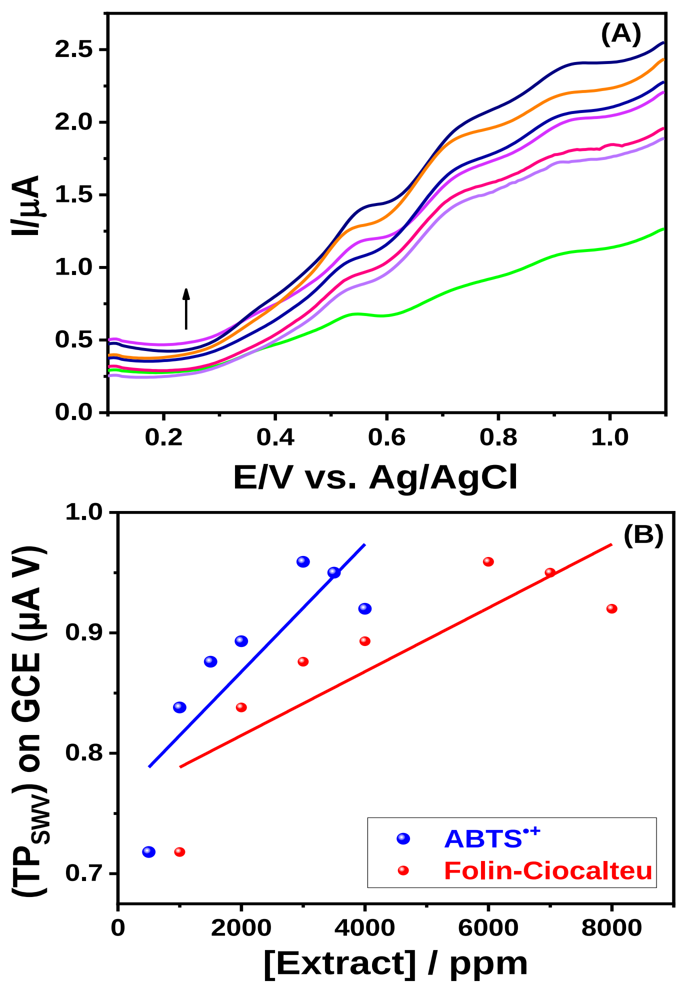

Fig. 4. (A) SWV curves of various additions of Juniper extract at GCE in B-R buffer solution of pH 2 at a frequency of 25 Hz, pulse amplitude of 20 mV, pulse width of 5 mV. (B) Correlation between total peak area of square wave voltammogram under the voltammogram (TP_{SWV} / μA V) *vs.* Folin Ciocalteu Index (FCI, mg Gallic Acid Equivalent (GAE) L^{-1}) and antioxidant activity determined by the ABTS method (mM GA Equivalent (GAE)).

4. Conclusion

The investigation into the antioxidant activities and polyphenolic contents of several medicinal herbs through voltammetric and colorimetric analyses has provided valuable insights into their chemical properties and health implications. The voltammetric data correlated well with the results from the colorimetric assays, indicating the efficacy of these methods in analyzing complex botanical matrices. The study confirmed the significant antioxidant potential of the herbs, attributable to their diverse polyphenolic compounds. This research enhances our understanding of the medicinal properties of these herbs, supporting their inclusion in dietary and therapeutic applications for their health-promoting benefits. Future studies should focus on isolating specific bioactive compounds and further elucidating their mechanisms of action in various biological systems.

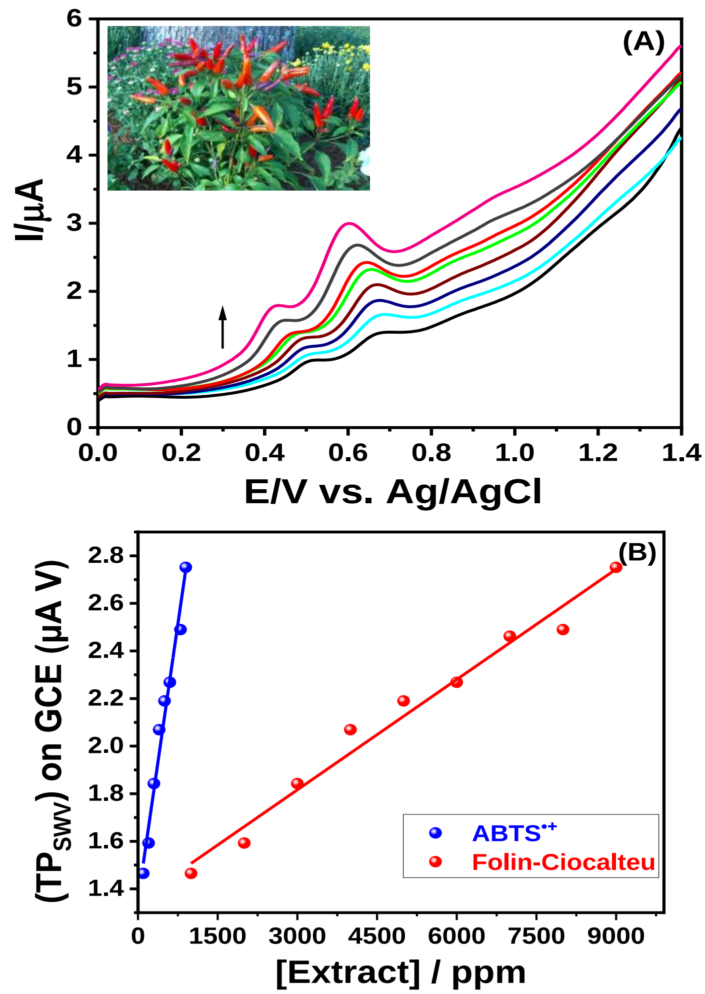

Fig. 5. (A) SWV curves of various additions of Bell Pepper extract at GCE in B-R buffer solution of pH 2 at a frequency of 25 Hz, pulse amplitude of 20 mV, pulse width of 5 mV. (B) Correlation between total peak area of square wave voltammogram under the voltammogram (TP_{SWV} / μA V) *vs.* Folin Ciocalteu Index (FCI, mg Gallic Acid Equivalent (GAE) L^{-1}) and antioxidant activity determined by the ABTS method (mM GA Equivalent (GAE)).

CRediT authorship contribution statement:

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Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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