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Analysis Insight of Egyptian *Ficus carica L.* Fruits Using Voltammetry and Ultra-high Performance Liquid Chromatography Coupled with Mass Spectrometry

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Abstract: Analysis of *Ficus carica L.* fruits using electrochemical technique and ultra-high performance liquid chromatography coupled with mass spectrometry (UPLC-MS) was carried out for the first time. The *Ficus carica*, commonly known as the fig tree, is a perennial fruit-bearing tree native to the Middle East and Western Asia and is considered one of the healthiest dried fruits because of its high mineral and vitamin contents. In the current manuscript, we will recognize and quantify the bioactive compounds that exist in *Ficus carica* fruits to provide valuable information for their potential therapeutic applications. Voltammetry and UPLC-MS techniques enable the identification and quantification of a wide range of bioactive antioxidant compounds found in complex matrices such as plant extracts. The analysis of *Ficus carica* fruits using UPLC revealed the presence of several electroactive species such as phenolic acids, flavonoids, and tannins. Interestingly, the SWV showed sensitive and reproducible results compared to the benchmark protocol for assessment of the antioxidant activity.
Keywords: Antioxidant activity, voltammetry, liquid chromatography, fig fruits, cyanidins

1. Introduction

Ficus carica L. is a Moraceae plant native to southwest Asia and the eastern Mediterranean. Fig trees are considered to be the first plants domesticated by humans, and they are now an important crop throughout the world [1]**.** Dried figs are one of the healthiest dried fruits because of their high mineral and vitamin contents [2]. It has been estimated that dried and fresh figs contain relatively high levels of crude fiber (5.5% w/w) and polyphenols [3,4]. In several studies, fig antioxidants protected lipoproteins from oxidation, and fig antioxidant consumption increased plasma antioxidant capacity for up to 4 hours afterward [4]. According to a published study, polyphenols, particularly anthocyanins, enhance the antioxidant activity of fig fruit [5]. As a result of a study conducted by Robinson and Robinson, cyanidin 3-glucoside was identified as a compound found in Figs [6]. The pigment also comprises approximately 75% cyanidin 3-rhamnoglucoside, 11% of cyanidin 3,5-diglucoside, 11% of cyanidin 3-glucoside, and 3% of pelargonidin 3-rhamnoglucoside [5,7,8]. To determine the anthocyanins, polyphenols, and flavonoids in figs, the pulp and skins of commercially grown figs are tested [5]. In contrast to fruits with lighter skins, fruits with darker skins contain a greater number of polyphenols and antioxidant activity. It is demonstrated that antioxidant-containing foods play a significant role in maintaining a healthy diet [9-11].

Among different analytical chemistry techniques, electrochemical tools have attracted much attention for the analysis of many analytes, including inorganic and organic species. The electrochemical methods have many advantages in terms of their speed, low-cost instrumentation, and affordability. Electrochemical methods have been used to measure the antioxidant capacity by several research groups [12-17]. A qualitative assessment of the sample's antioxidant content is also possible. Various antioxidant compounds, including phenols and ascorbic acid, exhibit different oxidation potentials, which are manifested on the voltammogram in different ways [18-20]. Although electroanalytical-based methods possess considerable potential, they are currently underutilized and understudied. Compared to other methods for antioxidant determination in the literature, electrochemical methods are used in fewer studies. It is, therefore, necessary to create a routine methodology through the use of multiple samples and distinct conditions [21-23].

The current manuscript aims to demonstrate the antioxidant properties and measure the antioxidant activity of fig fruit extract. Two major analytical techniques of ultra-high-performance liquid chromatography (UPLC) and voltammetry will be utilized. The UPLC will be used to identify the phenolic compounds that contribute to the fruit's antioxidant properties. While the total antioxidant capacity will be measured by electrochemical methods. Additionally, the nutritional value and antioxidant capability based on polyphenol content will be discovered.

2. Materials and methods

2.1. Chemicals

Cyanidin-3-*O*-rutinoside chloride (Cy 3-rtu, analytical standard), boric acid (≥ 99% ACS), phosphoric acid (49% HPLC), glacial acetic acid (USP), sodium hydroxide (≥ 98% reagent grade) were acquired from Sigma-Aldrich Co. LTD, France. Acetone (≥99.9% HPLC), ethanol (≥99.8% HPLC), and methanol (≥99.9% HPLC) were supplied from Merck (United States). Metrohm (France) provided the alumina powder. A

Milli-Q water system was used to obtain ultrapure water for preparing the solutions.

2.2. Fruit Sample Preparation

Local markets in Alexandria, Egypt, provided commercially available fig fruits for this study. The fruits were manually peeled (2–5 units), and the skin and pulp were separated. As the pulp (21 grams) was ground and homogenized, vigorous shaking was used to homogenize it in a MeOH solution containing 0.1% hydrochloric acid (HCl). The samples were centrifuged at 5000 rpm for 20 minutes to remove particulates. The extract was dried at 30°C in a rotator evaporator until the solvent volume was reduced. The final volume was adjusted to 20 mL of a 50:50 mixture of methanol and water.

2.3. Solutions

A 50:50 mixture of ethanol and water was used to prepare the stock solution of Cy 3-rtu (1.43 mmol L^{-1}). Britton-Robinson buffers are used as supporting electrolytes. A B-R buffer comprises phosphoric acid (0.04 mol L^{-1}), boric acid (0.04 mol L^{-1}), and acetic acid (0.04 mol L^{-1}). Ammonium hydroxide (0.2 mol L^{-1}) is added to adjust the pH to the desired value. After protecting the solutions from light with aluminum foil, all solutions were kept in a refrigerator at -5 °C. There was a five-week cycle for preparing stock solutions. UPLC-UV chromatograms were recorded at 520 nm every four days using the stock solutions injected into the UPLC-MS. A UPLC-UV chromatogram can be used to determine the stability of solutions based on the calculated peak area. Each time a new experiment was conducted, the solutions were freshly prepared from the stock solutions. The pH of the solution throughout the experiment was measured by a pH meter from HANNA Instruments.

2.4. Voltammetric Measurements

Voltammetric measurements were conducted with an Autolab PGSTAT128N Potentiostat/Galvanostat and NOVA 1.10 software from Eco-Chemie, Utrecht, Netherlands. An electrochemical cell containing three standard electrodes (20 mL) was used to conduct voltammetry experiments. The working electrode is a glassy carbon electrode (GCE) with a diameter of 3.0 mm supplied from Metrohm-Autolab, Switzerland, silver/silver chloride reference electrode (Ag/AgCl, aq. KCl, 3.0 M), and the counter electrode was platinum wire. The GCE surface was cleaned with 0.3 mm alumina powder, the surface was rinsed thoroughly with water, and then the electrode was immersed in an ultrasonic bath for five minutes before each measurement. Following this, GCE was maintained in a buffer solution consisting of B-R. Cyclic voltammetry measurements were performed until the electrochemical signal had been stabilized. Three consecutive measurements were performed to assess the reproducibility of the experimental results.

2.5. UPLC-DAD-MS system

Ultra-high-performance liquid chromatography connected with Mass spectrometry was utilized to determine the anthocyanins in fig fruits. The liquid chromatography system consists of an Acquity UPLC with a photodiode array detector from Waters in Milford, Massachusetts. The Nucleosil 120-3 C18 end-capped column (Macherey-Nagel, Sweden) was utilized in the experiment (HSS T3, 100 2.1 mm, 1.8 mm). With a flow rate of 0.55 mL min^{-1}, the gradient conditions were solvent A (H_2O/HCOOH, 99/1, v/v), solvent B (CH_3CN/H_2O/HCOOH, 80/19/1, v/v/v), and 0.1% B for initial, 60% B linear for 0–5 min, 99% B linear for 5-7 min, 99% B isocratic for 7-8 min, and 0.1% B linear for 8–9 min. A mass spectrometer called the amaZon X ESI-Trap from Bruker Daltonics in Bremen, Germany, was connected online to the Acquity UPLC system. In the source, the capillary voltage was set to 4 kV, the dry gas temperature was set to 200 °C with a flow rate of 12 L min^{-1}, and the nebulizer pressure was 44 psi. Positive ionization was used to gather the mass spectra in the 90–1500 Th mass range. The 8.1 m/z min^{-1} mass spectrum acquisition speed was chosen.

2.6. Determination of Antioxidant Activity (AA) by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Method

The AA was calculated by the ABTS protocol as suggested by Guedes et al. [24]. The ABTS radical cations ($ABTS^{\bullet+}$) were produced in a solvent mixture of ethanol/water (1:1, v: v) by mixing 7 mmol L^{-1} ABTS and 2.45 mmol L^{-1} $(NH_4)_2S_2O_8$ solutions. In the early stages of ABTS oxidation, the absorbance plateaus, but it takes some time before it reaches its maximum level. After 24 hours, the $ABTS^{\bullet+}$ solution was adjusted to achieve an absorbance of 0.7000±0.020 at 734 nm by diluting it in an ethanol/water solvent. After 6 minutes of processing, the absorbance of the fig extract was determined by combining 1 mL of diluted ABTS+ solution with 0.1 mL of diluted extract samples. JASCO International Co., LTD., Tokyo, Japan's V-750 ST UV-visible spectrophotometer and Spectra Manager 2 software were used to conduct the absorbance measurements.

3. Results and Discussion:

3.1. UPLC-MS Identification of Polyphenolic Compounds in Fig Fruit

Fig. 1 illustrates the UPLC pigment profile of an extract of the *Ficus carica* L. fig fruit pulp. According to UPLC-DAD-MS analysis, there is only one peak for anthocyanin in the pulp at 520 nm. Based on the mass spectral data and UV–vis spectra, the pigment was identified as Cyanidin-3-*O*-rutinoside (Cy 3-rut). It was recognized by comparing its chromatographic features and absorption spectra with those found in our library, and it was confirmed by mass spectral analysis of Cy 3-rut, which shows only a m/z 595 $[M-H]^+$. It was revealed that up to 12 polyphenols were present in the fig sample analyzed. A UPLC phenolic profile of pulp extract is shown in Fig. 2. According to Table 1, the retention time and molecular ion data obtained for the phenolic compounds were obtained from UPLC-DAD-MS analyses. Five phenolic compounds were identified at 280 nm. These were 9,12,15-octadecatrienoic acid (Peak 1), Cy 3-glucoside (Peak 3), Cy 3-rutionside (Peak 5), Epi (4-8) Pg 3-rutionside (Peak 6), and Luteolin C-hexoside C-pentoside I (Peak 8). Among the phenolic compounds found in fig pulp, Cy 3-rutionside (Peak 5) is the most abundant. As a result of comparing their chromatographic profiles and

absorption spectrums with those in the library and confirming their identity by mass spectrometry (Table 1), we could determine the identity of these compounds. Comparing our library of phenolic data with peaks 2, 4, 7, 9, 10, 11, and 12 did not identify these peaks. Additionally, the concentration of these compounds might have been below the detection limit of the analytical instrument used. UPLC, while highly sensitive, does have its limits in terms of the minimum concentration it can detect. If the compounds of interest were present in very low concentrations, they may have gone undetected during the analysis. Another factor that could have contributed to the inability to identify the compounds is the complexity of the samples themselves. In real-world samples, such as plant matrices, there can be a wide range of compounds present, each with varying chemical properties. This complexity can pose challenges for accurate identification, even with advanced techniques.

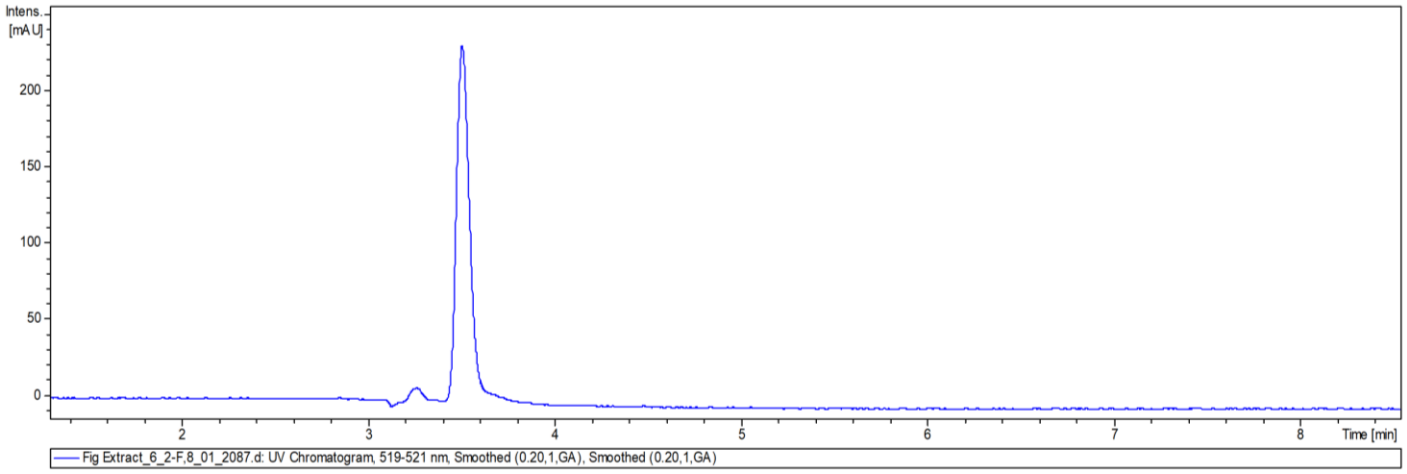


Fig. 1. UPLC Chromatogram recorded at 520 nm showing the anthocyanin profile of Egyptian *Ficus carica* L. (fig pulp) fruit.

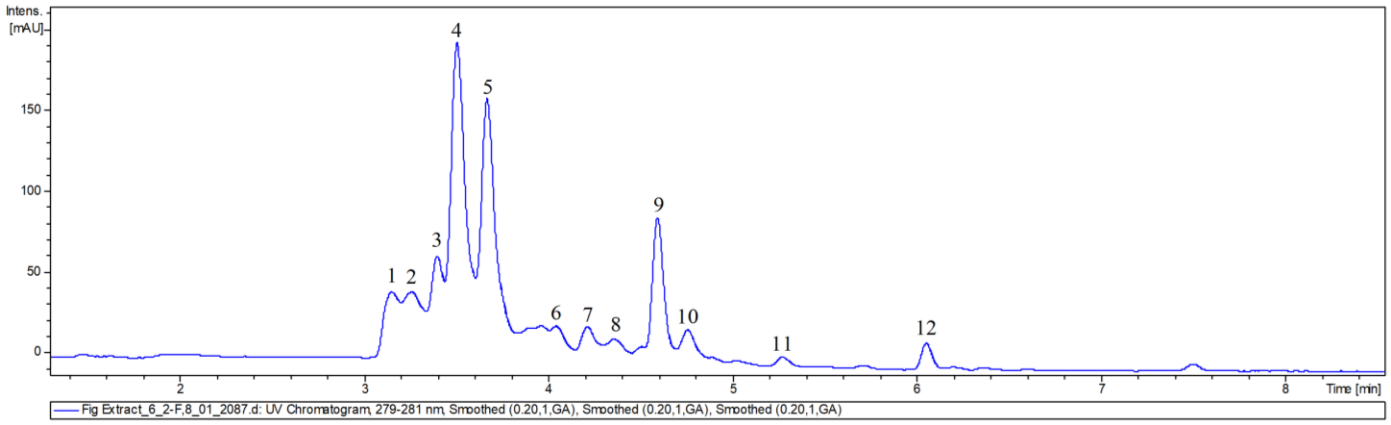


Fig. 2. UPLC Chromatogram recorded at 280 nm showing the total polyphenols profile of Egyptian *Ficus carica* L. (fig pulp) fruit.

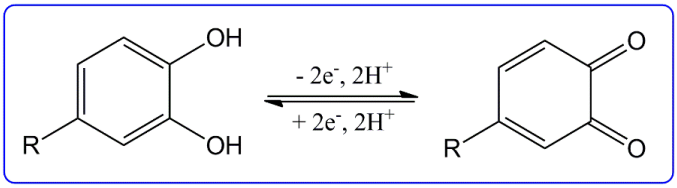
Table 1. Retention time (R_t), mass spectral data, and tentative identification of polyphenols in fig pulp at λ_{max} = 280 nm.

Peak	R_t (min)	Molecular ion $[M+H]^+$ (m/z)	Tentative Identification
1	3.1	280.9	9,12,15-octadecatrienoic acid
2	3.3	193.1	*Not identified*
3	3.4	449.1	Cy 3-glucoside
4	3.5	660.2	*Not identified*
5	3.7	595.2	Cy 3-rutionside
6	4.0	542.3	Epi (4-8) Pg 3-rutionside
7	4.2	455.3	*Not identified*
8	4.4	459.3	Luteolin C-hexoside C-pentoside I
9	4.6	569.2	*Not identified*
10	4.7	229.1	*Not identified*
11	5.3	247.1	*Not identified*
12	6.1	313	*Not identified*

3.2. Voltammetric Behavior of Polyphenolic Compounds in Fig Fruit

Polyphenols undergo oxidation reactions because they contain ortho-dihydroxyl groups in their structures. According to a previous study, catechol is oxidized through two-electron transfers followed by irreversible chemical reactions that produce *o*-quinone [25]. As can be seen in Scheme 1, catechols undergo an overall oxidation process that results in *o*-quinones. As a consequence, fig extract, which also contains a catechol moiety, will undergo oxidation on the surface of a glassy carbon electrode (GCE). A study was conducted at GEC to explore the electrochemical behavior of the standard Cy 3-rut, which is the major phenolic found in fig pulp. Utilizing SWV has several benefits over cyclic or differential pulse voltammetry, such as fewer electroactive species being used, analysis times are shorter, and there are fewer issues with electrode surface poising [26]. In addition, the current is sampled in both positive and negative pulses, electroactive species at the electrode surface can have their oxidation and reduction peaks identified in a single scan, and the reversibility of the electron transfer can also be assessed in a single scan. As a model compound among polyphenols in a B-R buffer (pH 2.2), preliminary studies were conducted by SWV at a glassy carbon electrode to investigate the electrochemical behavior of Cy 3-rut. In the B-R buffer (pH 2.2) with 70 µM Cy 3-rut, a redox peak was observed at E_p=+0.572 V *vs.* Ag/AgCl (Fig. 3). In this peak, the catechol moiety (3,4-dihydroxy substituents) is oxidized to the corresponding quinone structure. In a recent study [27], we showed that eriodictyol may be oxidized to produce *o*-quinone by using two electrons and two protons. Fig. 3 shows that the Cy 3-rut redox wave is reversible because the oxidation peak potential (E_p^a = +0.572 V) was near to the matching reduction peak potential (E_p^c = +0.567 V). Due to product adsorption, the electrochemical oxidation of Cy 3-rut can also result in electrochemical fouling. Due to the production of a polymer-like film [28] made of reactive side products and dimers, the oxidation of Cy 3-rut results in the formation of a porous film [29,30]. Using repeated SWV scans, 70 µM Cy 3-rutin B-R buffer (pH 2.2) was evaluated for oxidation over a potential range of -0.2-1.5 V *vs.* Ag/AgCl (Fig. 4). Upon repeated SWV scanning, the faradaic peak current decreases due to a porous insulating layer forming on the electrode surface [28], which reduces the flux to the electrode surface. The voltammograms displayed a drop in the first scan, followed by a slower decline according to peak current data (Fig. 4). There are several possible reasons for the decrease in peak current. These include reductions in electrode area, reductions in available adsorption sites for Cy 3-rut, and the possibility of the partitioning of Cy 3-rut within the polymer film, etc. [28]. With this data, electrode fouling can be measured in an additional way, and real-time monitoring of the electrode condition can be in-situ explored. By observing the decrease in peak current, it may be possible to determine whether it is related to a change in the Cy 3-rut concentration or to electrode fouling.

To quantify the total polyphenolic content (TPC), the SWV of the fig extract was measured to explore the analytical potential of electrochemical oxidation of electroactive phenolic species present in the extract. The polyphenolics in the extract will oxidize as the potential of the GCE is swept in a positive direction during the SWV measurements. Based on oxidation waves of various polyphenolic species existing in the fig extract, the overall response to the current was calculated.



Scheme 1. The overall oxidation reaction of phenolic compounds.

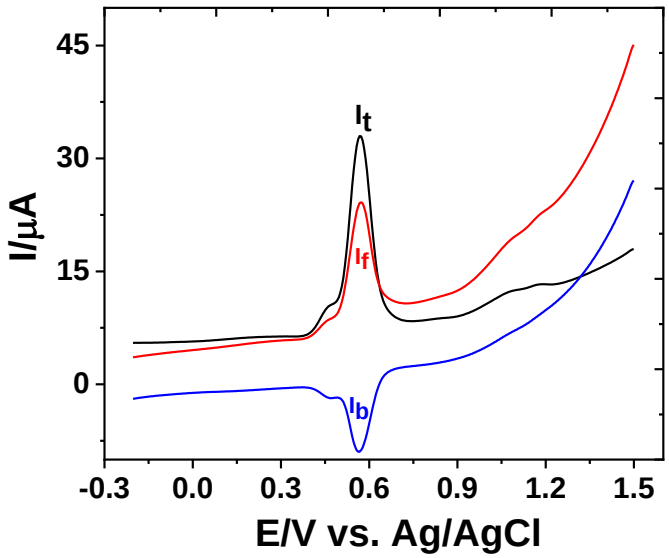


Fig. 3. SWV of standard polyphenol 70 µM Cy 3-rut on GCE in B-R buffer solution of pH 2.2, I_t is the total current, I_f is the forward current, I_b is the backward current at a frequency of 10 Hz, pulse amplitude of 25 mV, and pulse width of 1.0 mV.

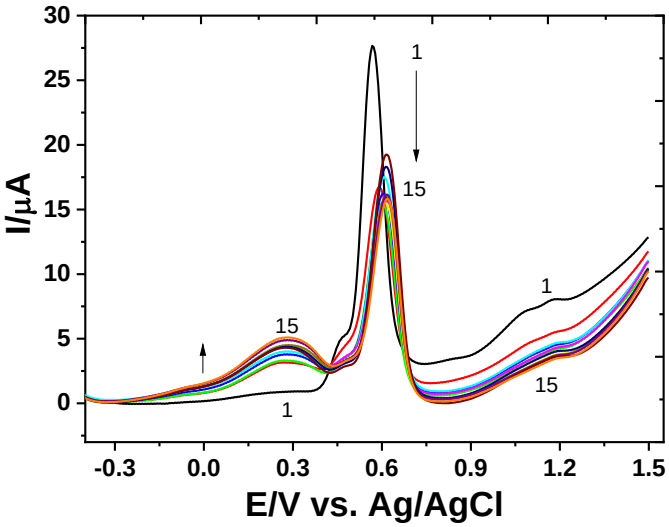


Fig. 4. SWV curves of 70 µM Cy 3-rut in B-R buffer solution of pH 2.2 at GCE for 15 cycles without polishing the electrode at a frequency of 10 Hz, pulse amplitude of 25 mV, and pulse width of 1.0 mV.

Fig. 5 illustrates the SWV of four dilutions of the prepared fig extract at GCE in a B-R buffer solution of pH 2.2. There is an oxidation wave at E_p^a = +0.501 V, which is because of the

oxidation of 3,4-dihydroxy substituents. The results obtained confirm that Cy 3-rut is the predominant polyphenol in fig extract since they are so similar to those obtained for the standard Cy 3-rut sample. A further measure of the response of the total polyphenols (TP_{SWV}) in the fig extract can be obtained by calculating the area under the voltammetric signal for the SWV measurements. As a result of the signal of several polyphenols with slightly different oxidation potentials, this measurement is more appropriate, especially for a complex fruit matrix, and gives information about the total amount of polyphenols in a fruit. A linear shift in the TP_{SWV} was observed as the concentration of extracts increased. For the investigated fig fruit, linear regression equations were obtained as follows: TP_{SWV} (µA V) = $6.09 \times 10^{-8} + 5.84 \times 10^{-8}$ C (% v/v), $r^2 = 0.963$. As a result of the following section, the TP values gained by SWV (TP_{SWV}) will be correlated with those attained from antioxidant activity measurements (AA_{ABTS}).

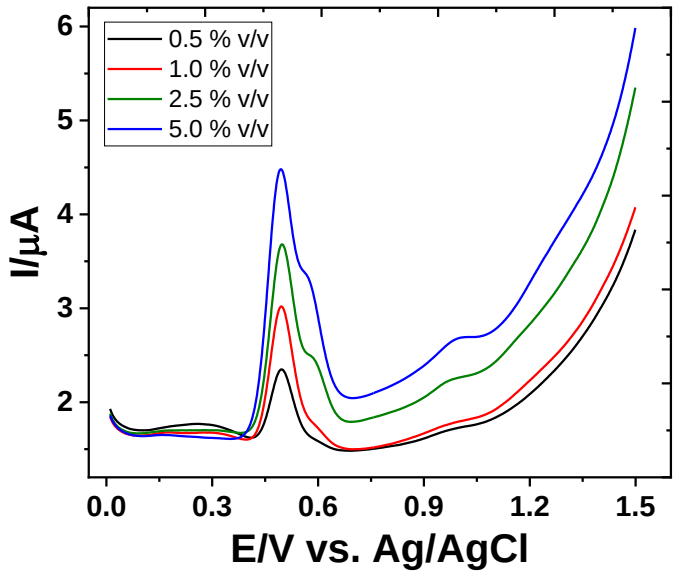


Fig. 5. SWV curves of various additions of fig extract at GCE in B-R buffer solution of pH 2.2 at a frequency of 10 Hz, pulse amplitude of 25 mV, pulse width of 1.0 mV.

3.3. Antioxidant Activity using ABTS measurements

Fig extract was evaluated for its ability to scavenge free radicals using stable ABTS radicals. In terms of Trolox Equivalent Antioxidant Capacity (TEAC), antioxidative activity (AA_{ABTS}) was expressed in a mole of TE per Liter. For the diluted extract samples, the diminution in absorbance ($DA=A_{ABTS} - A_{extract}$) would be calculated. To determine the calibration curve for diminution in the absorbance value ($DA=A_{ABTS} - A_{Trolox}$) of Trolox *vs.* Trolox concentrations, we performed a similar procedure for Trolox concentration in the range of 50-600 mmol L^{-1}. Fig. **6A** shows the calibration curve for Trolox concentrations *vs.* the diminution in absorbance value. There is a positive correlation between total phenolic compounds in fig extract (TP_{SWV}, µA V) and their antioxidant activity (AA_{ABTS}) using SWV (Fig. **6B**). The value of the correlation coefficient (r^2) for the fig extract components was found to be 0.967. As a result of these findings, it can be concluded that phenolic compounds, which are the significant components of AA, are extremely significant to the antioxidant activity of fig extract as well as contributing greatly to the total antioxidant capacity. There is an excellent correlation between the concentration of polyphenols and the ability to scavenge radicals. According to our findings, the Egyptian fig was a rich source of phenolic constituents and demonstrated a high level of AA and the simple electrochemical technique can be a suitable and reproducible tool for estimating the antioxidant activity. Therefore, quantitative, and qualitative analyses of foremost individual phenolics in these kinds of fruits may be useful for demonstrating the relationship between total antioxidant capacity and total phenolic content in these kinds of fruits.

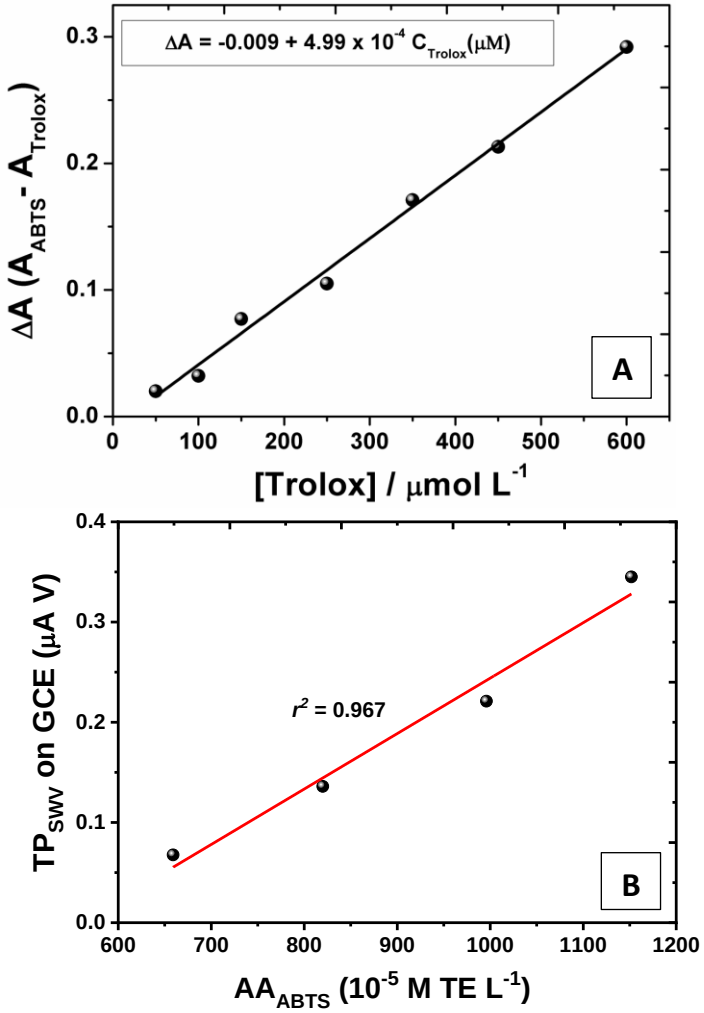


Fig. 6. (A) Calibration curve constructed from the difference in Absorbance at 734 nm vs. Trolox concentrations, (B) The relation between total polyphenols content (TP_{SWV}) calculated from the total peak area of SWV at GCE (µA V) vs. AA which determined by ABTS protocol (AA_{ABTS}, mol Trolox Equivalent (TE) L^{-1}) of fig extract.

4. Conclusion

The existence of various phenolic compounds in food that have interesting antioxidant activity suggests potential health benefits, anti-inflammatory, and cardioprotective effects. Analysis of *Ficus carica* fruits using simple electrochemical-based technique and ultra-high performance liquid chromatography coupled with mass spectrometry were performed to provide valuable insights into the bioactive

composition and possible voltammetric analysis because the *Ficus carica* fruits and their bioactive compounds are valuable for the development of novel pharmaceuticals or functional food products. Thus, the bioactive antioxidant compounds in figs were qualitatively and voltammetric quantitively determined using UPLC-MS and SWV. The results showed reproducible results compared to the benchmark antioxidant measurement activity method.

CRediT authorship contribution statement:

Emad F. Newair: Conceptualization, Investigation, Data curation, Validation Writing – original draft, Writing – review & editing, Project administration. *Aboelhasan G. Shehata:* Investigation, Methodology, Data curation. *Mohamed Khairy:* Conceptualization, Supervision, Writing – review & editing, Project administration.

Data availability statement

The corresponding author can provide data supporting the findings of this study upon request.

Declaration of competing interest

The authors declare that they do not have any competing financial interests or personal relationships that could have appeared to influence the findings reported in this paper.

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