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Determination of Antioxidant Capacity of *Solanum aethiopicum L.* Fruit Extracts Using Spectroscopic and Voltammetric Methods

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ABSTRACT

Antioxidants are compounds that neutralize free radicals and are essential for human health. *Solanum aethiopicum*, a specie of flowering plant within the *Solanaceae* family, is rich in bio-active compounds. We studied the total phenolic content, total flavonoid content, and antioxidant capacity of six *S. aethiopicum* cultivars (Shum group EP1, Gilo group EP2, Kumba group EP3, Gilo group2 EP4, Kumba group2 EP5, and Kumba group3 EP6) using spectroscopic (ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH)) and cyclic voltammetric assays. Fruit extracts of *S. aethiopicum* were obtained via microwave-assisted extraction utilizing methanol and n-hexane as solvents. The results indicated higher flavonoid and phenolic concentrations in the extracts. DPPH assay exhibited superior radical scavenging inhibition efficiency in methanolic compared to n-hexane extracts. Similar trend was observed in the FRAP assay. Cyclic voltammetric analysis revealed that the antioxidant capacity of the methanolic extracts was higher in EP2 (7.40±0.11 µgAA Eq/mg and 3.11±0.07 µgGA Eq/mg) and lower in EP4 (2.95±0.15 µgAA Eq/mg and 0.38±0.09 µgGA Eq/mg). The findings of this work suggest that *S. aethiopicum* is good source of bioactive compounds with potent antioxidant capacity. Integration of spectroscopic and electrochemical techniques represents a promising methodologies for assessing the antioxidant capacity of fruit extracts.

Keywords: Antioxidant, cultivar, spectroscopy, *Solanum aethiopicum*, voltammetry

INTRODUCTION

Fruits are good sources of bioactive compounds, which are beneficial for human health, particularly those that have good pharmacological properties [1 - 2]. The imbalance between free radicals and antioxidants in the body causes oxidative stress, which contributes to many chronic illnesses, including diabetes, cancer, heart disease, and neurological disorders [2 - 4]. Consequently, a significant area of focus in food, pharmaceutical, and biological research is focused towards the identification and characterization of plant-derived antioxidants due to

their minimal side effects compared to synthetic antioxidants. The strong antioxidant properties of phenolic and flavonoid compounds make them among the most researched classes of phyto-chemicals.

Solanum aethiopicum L. (*S. aethiopicum*), commonly known as African eggplant, is a specie of flowering plant belonging to the *Solanaceae* family. There are over 1000 species globally, and at least 100 of them can be found in Africa and nearby islands [1, 5]. About 25 species have been domesticated in Nigeria, prominent among these include *S. melongena* (aubergine), *S. macrocarpon L.* (Gboma eggplant), and *S. aethiopicum L.* (Ethiopian eggplant) [1]. In Nigeria, *S. aethiopicum L.* is locally known as "gauta" in Hausa [6], "igbagba" in Yoruba, and "afufa or añara" in Igbo languages, respectively. *S. aethiopicum* was earlier classified into four distinct species, namely: Gilo, Shum, Kumba, and Aculeatum, which are all now referred to as cultivars of *S. aethiopicum* [7 - 8]. Each of these cultivars is cultivated for a specific purpose of the plant parts. For example, the Gilo and Kumba are commonly planted for their fruits both in rural and urban areas and mostly consumed raw or cooked in stews [9]. The Shum group is cultivated for its leaves and eaten just like spinach; the aculeatum group, on the other hand, is cultivated for its rhizomes and ornamental purposes [8 - 9]. *S. aethiopicum* contains macronutrients, essential vitamins [10 - 11] and has excellent medicinal applications [10, 12 - 15].

Spectroscopic assays such as ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), the scavenging of 2,2'-diphenyl-1-picrylhydrazyl free radicals, and Trolox equivalent antioxidant capacity (TEAC) are the most frequent assays employed in the literature for the determination of antioxidant capacity of several matrices [16 - 17]. However, these techniques are associated with drawbacks such as time-consuming during sample preparation, high equipment costs, the need for specialized training to operate the equipment, poor sensitivity and selectivity, interference from colour and turbidity in the samples [16,18]. To address some of the challenges of spectroscopic assays, electrochemical method has emerged as an alternative technique for determining the antioxidant capacity of plant matrices owing to its simplicity and cost-effectiveness. Electroanalytical techniques such as cyclic voltammetry and chronoamperometry have been reported in the literature for this purpose [17, 19 - 24].

S. aethiopicum fruit contains a variety of bioactive compounds such as flavonoids, phenols, etc. To the best of our understanding, only the spectroscopic method [6, 25 - 27] has

been used to evaluate the antioxidants capacity of *S. aethiopicum*. There is no report of using electrochemical methods to evaluate the antioxidant capacity of the fruits of *S. aethiopicum*. Therefore, the present study explores the use of both spectroscopic (DPPH and FRAP) and electrochemical (cyclic voltammetry) methods to determine the antioxidant capacity of six varieties of *S. aethiopicum L*. (Plate 1). The total flavonoid and phenol contents of *S. aethiopicum L*. are also evaluated.

Plate 1: Fruit samples of *Solanum aethiopicum L*. subgroups. **(a)** Shum Group, EP1 **(b)** Gilo Group, EP2 **(c)** Kumba Group, EP3 **(d)** Gilo Group, EP4 **(e)** Kumba Group, EP5 **(f)** Kumba Group, EP6

MATERIALS AND METHODS

Sample collection and preparation

The different *Solanum Aethiopicum* fruit species were purchased from Central Market in Kano, Kano state, Nigeria, and were identified by a botanist at the Herbarium in the Department of Plant Science and Biotechnology, Bayero University, Kano State, Nigeria. The fruits were washed thoroughly with water, cut into smaller pieces, and allowed to dry inside a laboratory before grinding into a powder using a conventional mortar and pestle.

Preparation of fruit extracts of *Solanum aethiopicum*

The microwave-assisted extraction of *Solanum Aethiopicum* extracts was carried out as described in the literature [4, 28] with slight modifications using a conventional microwave oven (Daewoo, KOC-9Q4T) with a variable power and time controller. About 100 g of

powdered fruit sample of *Solanum Aethiopicum* was weighed into a Bama bottle, and 200 mL of n-hexane was added and allowed to stand for about 30 min. The bottle was covered tightly and irradiated using the microwave oven at a temperature of 150 °C and set at defrost mode, microwaved 5 times, each for 3 min, removed, and allowed to cool in between the pulses. The mixture was washed using n-hexane and filtered using a muslin cloth. This procedure was repeated using a methanol/water mixture in the ratio of 70:30. The extracts obtained were then concentrated using a rotary evaporator (BUCHI R-210, Switzerland) at 40 °C and subsequently dried at room temperature.

Determination of total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu method [29 - 30]. Briefly, 0.1 mL of the extract was added to a 0.1 mL Folin-Ciocalteu reagent in a volumetric flask, and additional distilled water was added to make a final volume of 10 mL. A reagent blank was prepared using distilled water. Folin-Ciocalteu phenol reagent (0.5 mL) was added to the mixture and shaken vigorously. After 5 min, 5 mL of 5% Na_2CO_3 solution was added, and the mixture was thoroughly mixed. The solution was immediately diluted to 25 mL with distilled water, mixed thoroughly, and then allowed to stand for 20 min. The absorbance was measured at 760 nm versus the prepared blank. The total phenolic content of the sample was expressed as gallic acid (GA) equivalent (Eq) per milligram (mg) of the extract using the calibration curve.

Determination of flavonoid content

The total flavonoid content was measured as described in the literature [30 - 32]. About 40 µL of *Solanum Aethiopicum* extract was diluted with 1.25 mL of distilled water and mixed with 15 µL of $NaNO_2$ solution. After 5 min, 30 µL of $AlCl_3$ solution was added and then incubated for 10 min, and 50 µL of 1 M NaOH solution was subsequently added. The absorbance was measured immediately at 510 nm. The total flavonoid content of the extract samples of *Solanum Aethiopicum* was expressed as Quercetin (Qc) equivalent (Eq) per milligram (mg) of the extract using the calibration curve.

Analyses of antioxidant capacity

DPPH radical scavenging activity

Antioxidant activity was determined using the DPPH assay [30, 33 - 34]. The reaction mixture (3.0 mL) consists of 1.0 mL of DPPH in methanol (0.3 mM), 1.0 ml of extract, and 1.0 mL of

methanol. The absorbance was measured spectrophotometrically at 517 nm. The percentage of inhibition was calculated using equation (1).

$$\%\text{Inhibition} = \frac{AC - AS^{*}}{AC} \times 100 \quad (1)$$

AS* = AS - AB, AS = Absorbance of the sample + DPPH, AB = Absorbance of the sample only, AC = Absorbance of DPPH only.

Ferric reducing antioxidant power

The reducing potential of the fruit extract was carried out as previously described in the literature [30, 35 - 36]. Different concentration of extracts were prepared, and the total volume of the solution was maintained at 400 µL. Each sample was mixed with 1 mL of phosphate buffer (0.3 M, pH 6.6) and 1 mL of 1% potassium ferricyanide ($K_3[Fe(CN)_6$) and incubated at 50 °C for 20 min. Then, 0.5 mL trichloroacetic acid (TCA) was added. 2 mL of the solution was mixed with 2 mL of distilled water and 0.2 mL of $FeCl_3 \cdot 6H_2O$ solution. This was allowed to stand for 10 min for colour development, and absorbance was measured at 700 nm using a spectrophotometer. The reducing power of the *Solanum aethiopicum* extract was determined using equation 2.

$$\%\text{FRAP} = \frac{A_a - A_b}{A_a} x100 \quad (2)$$

Where, A_a and A_b is the absorbance of the extract and blank, respectively.

Analysis of antioxidant capacity by cyclic voltammetric technique

The electrochemical experiment was carried out using the cyclic voltammetric method in a 10 mL glass electrochemical cell with three electrodes consisting of a glassy carbon disc (working electrode, 3 mm in diameter), a platinum wire as a counter electrode, and an Ag/AgCl (saturated KCl) as a reference electrode. Before usage, the glassy carbon electrode was polished using 0.3 µm alumina powder slurry. The potential of the working electrode was scanned in the range of 0 to +1.3 V vs Ag/AgCl at a scan rate of 50 mV/s. All measurements were carried out in triplicate using an open-source portable potentiostat (Rodeostat, RSTAT-01, USA). The antioxidant capacity of the *Solanum Aethiopicum* fruit extract samples was obtained from calibration curves using equation 3 with ascorbic and gallic acids as standards [29 - 30, 37 - 38].

$$\text{AC } (\mu g/mg) = \frac{C_{eq}}{C} \quad (3)$$

where AC is antioxidant capacity, C_{eq} is the equivalent concentration of gallic acid or ascorbic acid, and C is the initial concentration of the extracts.

RESULTS AND DISCUSSION

The present study assessed the antioxidant capacity, total phenolic content, and total flavonoid content of six varieties of *Solanum aethiopicum L.* cultivars using spectrophotometric and cyclic voltammetric methods.

Total phenolic contents

The total phenolic contents of the n-hexane and methanolic extracts of *S. aethiopicum* were determined using Folin-Ciocalteu reagent. Figure 1a depicts the results expressed in microgram (µg) of gallic acid (GA) equivalent (Eq) per milligram (mg) of extract (µg GA-Eq/mg). As presented in the Figure 1, the n-hexane extract shows a slightly higher phenolic content than the methanolic extract, except for the methanolic extract of EP4, which has the highest phenolic content value of about 169.49 µg GA-Eq/mg. The high phenolic contents in most of the n-hexane extracts may be attributed to differences in solvent polarity, with the phenolic compounds in the fruit extracts being more soluble in n-hexane than in methanol. The findings suggest that solvent polarity significantly influences phenolic extraction efficiency. Based on the results, the six species of the *S. aethiopicum* showed different phenolic levels in the following orders; EP6 (146.74 µg GA-Eq/mg) > EP2 (130.25 µg GA-Eq/mg) > EP1 (128.05 µg GA-Eq/mg) > EP3 (123.94 µg GA-Eq/mg) > EP5 (113.52 µg GA-Eq/mg) > EP4 (109.27 µg GA-Eq/mg) and EP4 (169.49 µg GA-Eq/mg) > EP6 (126.26 µg GA-Eq/mg) > EP5 (111.21 µg GA-Eq/mg) > EP3 (105.30 µg GA-Eq/mg) > EP1 (100.19 µg GA-Eq/mg) > EP2 (82.11 µg GA-Eq/mg) in the n-hexane and methanolic extracts, respectively. As observed, the phenolic content varies significantly among the six cultivars. Similar findings have been reported by other authors on different eggplant cultivars [39]. Several factors influence the phenolic content in fruits and vegetables, including cultivars, environmental conditions, soil type, growing and storage conditions, and extraction methods [40 - 41].

The total flavonoid content (TFC) was expressed in terms of microgram (µg) of quercetin (Qc) equivalent (Eq) per milligram (mg) of extract (µg Qc-Eq/mg). The six cultivars of *S. aethiopicum L.* showed different levels of flavonoid content (Figure 1b).

The methanolic extracts exhibited higher total flavonoid content than n-hexane extracts across all the *S. aethiopicum* cultivars, except for EP1 and EP3, where they exhibited higher flavonoid values of 222.20 and 150.33 µg Qc - Eq/mg, respectively. The findings suggest that flavonoid compounds in the fruit extracts of *S. aethiopicum* are more soluble in methanol than in n-hexane.

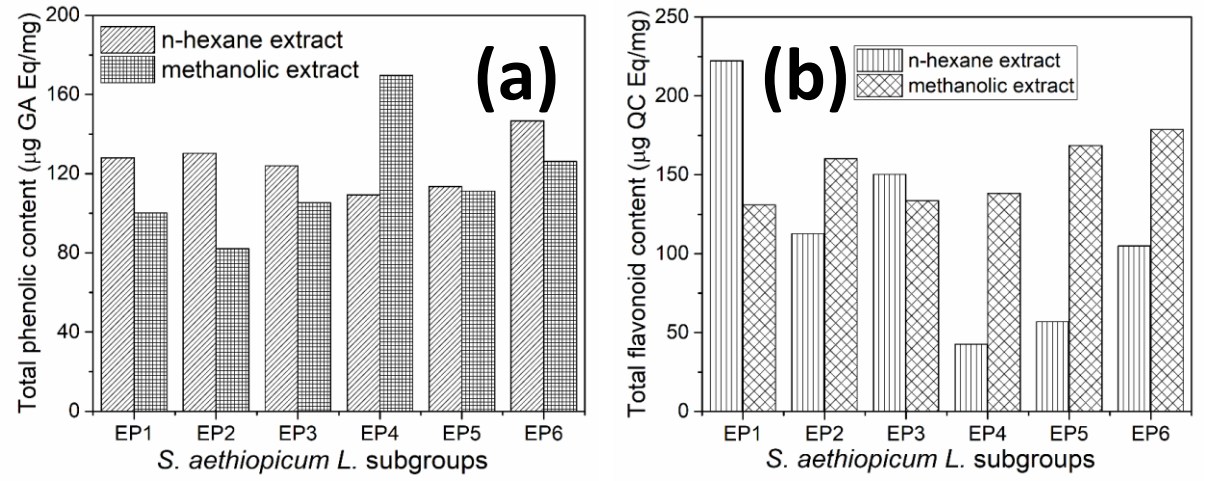

Figure 1. Total phenolic content (a) and total flavonoid content (b) of *Solanum aethiopicum L.*

The methanolic extract of EP6 had the highest flavonoid content (178.67 µg Qc - Eq/mg), followed by the methanolic extract of EP5 (168.53 µg Qc - Eq/mg) and then EP2 (160.13 µg Qc - Eq/mg). The flavonoid contents in the present work are significantly higher than those reported in other studies [21, 23, 42]. Plant sources and solvents used during extraction have been reported to affect the yield of flavonoid compounds [43].

Determination of antioxidant capacity using spectroscopic method

FRAP assay

The antioxidant capacity of fruit extracts of *S. aethiopicum* was determined using the FRAP assay. Ferric-reducing antioxidant potential analysis is based on the ability of phenolic and flavonoid compounds to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), forming a blue complex [44]. The reducing power of the methanolic and n-hexane extracts of *S. aethiopicum* cultivars is shown in Figure 2a and 2b, respectively. As shown in Figure 2a and 2b, the results obtained ranged from 22% to 106% and from 25% to 155% for n-hexane and methanolic extracts, respectively. Methanolic extracts demonstrated superior ferric-reducing antioxidant power compared to the n-hexane extract. Among the methanolic extracts, EP6 consistently displayed the highest reducing ability, especially at higher concentrations, whereas the n-hexane extracts overall exhibited weak metal-reducing power. Both the n-hexane and methanolic extracts consistently show an increase in reducing power with an increase in the concentration of the *S. aethiopicum* fruit extracts.

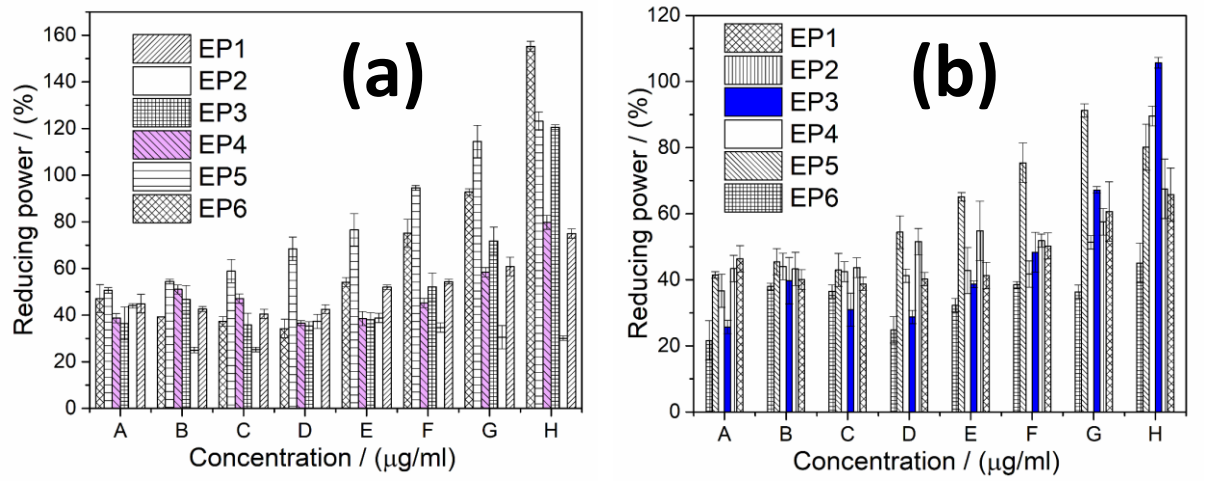

Figure 2. Reducing power of methanolic extracts (a) and n-hexane extracts (b) of *S. aethiopicum* cultivars (A=7.8125 µg/mL; B=15.625 µg/mL; C=31.25 µg/mL; D=62.50 µg/mL; E=125.00 µg/mL; F=250.00 µg/mL; G=500.00 µg/mL; H=1000.00 µg/mL)

DPPH assay

DPPH is a free radical species that has been widely used to probe the antioxidant activities of different plant samples, and the method is particularly sensitive to compounds that can act as hydrogen or electron donors. To evaluate the scavenging effect of DPPH on the methanolic and n-hexane extracts of *S. aethiopicum* fruit extracts, DPPH inhibition was carried out, and the results obtained are presented in Figure 3a and Figure 3b, respectively. As shown in Figures 3a and 3b, the free radical scavenging activities of the extracts of *S. aethiopicum* increased with increasing concentration. Similar results have been reported by other authors in the literature for other plant extracts [6, 45]. The methanolic extracts showed strong radical scavenging activity compared to the n-hexane extracts across all six cultivars and concentration levels. Within the methanolic extracts, EP5 demonstrated the highest scavenging activity (90.29% at 1000 µg/ml), indicating a strong antioxidant potential. On the other hand, the n-hexane extract of EP6 produced the highest scavenging activity (81.50% at 1000 µg/ml). The difference in the scavenging activity further suggests that the fruit extracts may contain different quantities of phenolic and flavonoid compounds that are either effective electron donors or hydrogen donors. Similar DPPH radical scavenging activity of *S. aethiopicum* was reported by some authors [6].

The results obtained from the FRAP measurement are higher than those from the DPPH method. The difference lies in the mechanism of action of the assay. DPPH assay involves measuring the ability of an antioxidant compound to donate either hydrogen or an electron that

will neutralize the DPPH free radicals and result in a change of color from purple to yellow due to the formation of diphenylpicrylhydrazine. On the other hand, the FRAP assay evaluates the capacity of antioxidants by reducing ferric ions to ferrous ions. Therefore, it can be suggested that the bioactive compounds in the plant extracts of *S. aethiopicum* are more effective in metal ion reduction than in free radical scavenging.

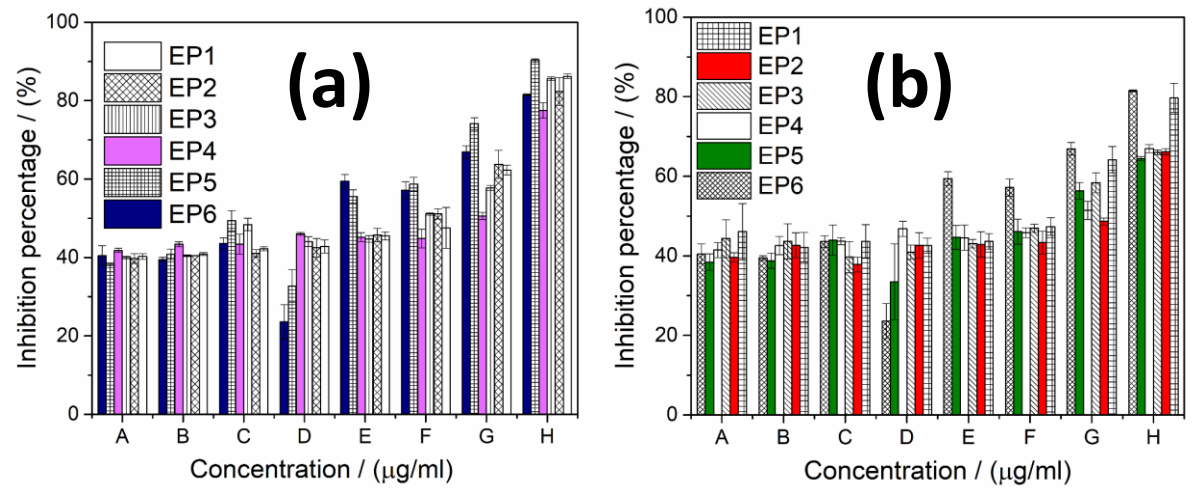

Figure 3. Variation of the percentage inhibition of DPPH of methanolic extracts (a) and n-hexane extracts (b) of *S. aethiopicum* cultivars (A=7.8125 µg/mL; B=15.625 µg/mL; C=31.25 µg/mL; D=62.50 µg/mL; E=125.00 µg/mL; F=500.00 µg/mL; G=1000.00 µg/mL)

Evaluation of antioxidant capacity by the cyclic voltammetry technique

Cyclic voltammetry is among the electrochemical techniques that are widely employed to evaluate the antioxidant capacity of plant and fruit extracts due to its simplicity of operation [46]. Peak current and potential are the most vital parameters needed to probe the antioxidant capacity of the extracts. The antioxidant and redox properties in fruit and plant extracts are mainly due to the presence of electrochemically oxidizable and reducible functional groups present in bioactive compounds such as phenols, flavonoids, vitamin C, and anthocyanins, among others. In the present study, only the methanolic extracts were used for the voltammetric analyses. All n-hexane extracts produced an oily product for the various fruit samples and were not used for the voltammetric measurement. Antioxidant capacity in the current work is reported in terms of equivalent standard ascorbic acid (AA) and gallic acid (GA). To obtain a calibration curve for the two standards, cyclic voltammetry measurements of different concentrations (0.2 – 1.8 mM) of AA and GA were recorded in 0.1 M phosphate buffer solution electrolyte containing 0.1 M NaCl. Figure 4 shows the cyclic voltammograms obtained for the

various concentrations of AA and GA at a scan rate of 50 mV/s. As seen in Figure 4, the voltammograms showed one irreversible oxidation peak for AA at ~0.2 V *vs* Ag/AgCl (Fig. 4a), and two oxidation peaks for GA at ~0.3 V and ~0.7 V *vs* Ag/AgCl (Fig. 4c). Similar voltammetric responses for AA and GA have been widely reported by other authors in the literature [45, 47]. In each case, the peak current increases as the concentration of the AA and GA was increased from 0.2 mM – 1.8 mM. The plots of the peak current vs concentrations of AA and GA are shown in Figures 4b and 4d, respectively, with all the plots showing linear behaviour. The linear regression equation of the calibration plot was obtained as I(μA) = 35.863 [AA] mM - 9.6183 and I(μA) = 59.35 [GA] mM + 4.3151, and the correlation coefficient value of R^2 = 0.971 and 0.947, for the AA and GA standards, respectively.

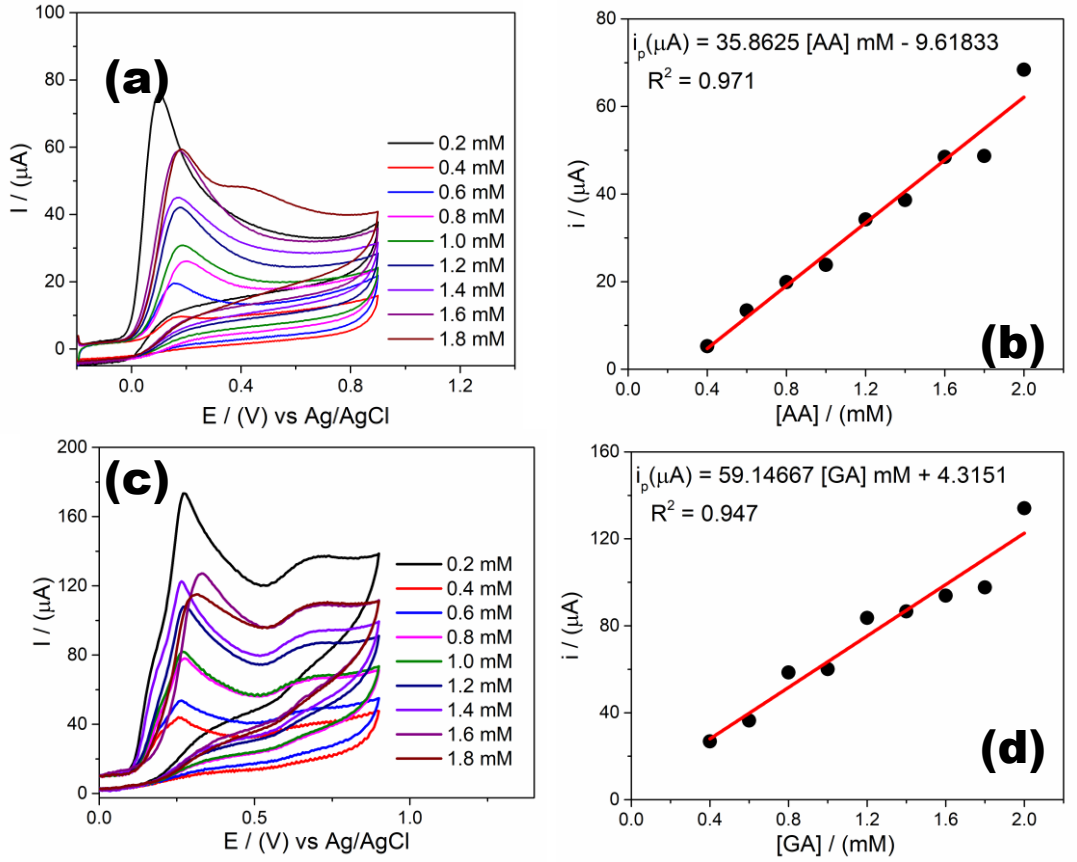

Figure 4. Cyclic voltammograms of different concentrations of ascorbic acid (a) and its calibration curve (b), gallic acid (c), and its calibration curve (d) recorded in 0.1 M phosphate buffer solution containing 0.1 M NaCl at a scan rate of 50 mV/s.

Cyclic voltammograms of methanolic extracts of different cultivars (EP1 – EP6) of *S. aethiopicum L*. recorded in a phosphate buffer solution as a supporting electrolyte are presented in Figure 5. All the voltammograms exhibited only a single anodic peak with a peak potential at ~0.76 V, ~0.76 V, ~0.74 V, ~0.74 V, ~0.76 V, and ~0.95 V *vs* Ag/AgCl for EP1, EP2, EP3, EP4, EP5, and EP6, respectively. Earlier studies indicate that some antioxidants, such as ascorbic acid, exhibit only one oxidation peak [48] while others, like gallic acid, Trolox, quercetin, and catechin, produce two oxidation peaks [49]. Hence, the result of voltammograms obtained in the current study could be related to the electrochemical behaviour of antioxidant compounds that have one oxidation peak, such as ascorbic acid. Peak potential values reveal the electron-donating power and reducing capacity of the different bioactive compounds in fruit extracts; a lower anodic peak potential indicates greater reducing power and vice versa [49]. EP1, EP2, and EP5 exhibited similar (~0.76 V *vs* Ag/AgCl) peak potential; EP3 and EP4 also showed the same value (~0.74 V *vs* Ag/AgCl), which suggests the presence of similar antioxidant compounds in the extracts.

The oxidation potential of EP6 is obtained at a slightly higher potential (~0.95 V *vs* Ag/AgCl) than the other cultivars, indicating that the extract may likely contain different bioactive compounds [24]. As shown in Figure 5, all extracts exhibit an irreversible behaviour due to the absence of the cathodic peak during the reverse scan. Similar irreversible electrochemical behaviour was observed for *thymus vulgaris* extract [21] and *Momordica charantia*, *Trichosanthes cucumerina* extracts [30]. The peak current is proportional to the concentration of the bioactive compounds that are present in the extracts. The area of the anodic peak current can be used to determine the antioxidant capacity of the extract [20, 50]. The antioxidant capacities of the methanolic extracts of *S. aethiopicum* determined using the standard calibration curves are in the order EP2 (7.40±0.11 µg AA Eq/mg and 3.11±0.07 µg GA Eq/mg) > EP6 (6.58±0.10 µg AA Eq /mg and 2.57±0.06 µg GA Eq /mg) > EP3 (6.12±0.22 µg AA Eq /mg and 2.30±0.14 µg GA Eq/mg) > EP5 (5.91±0.11 µg AA Eq /mg and 2.17±0.12 µg GA Eq/mg) > EP1 (4.21±0.10 µg AA Eq/mg and 1.21±0.16 µg GA Eq/mg) > EP4 (2.95±0.15 µg AA Eq/mg and 0.38±0.09 µg GA Eq/mg). The difference in the antioxidant capacity of the various *S. aethiopicum* extracts indicates differences in the composition and contents of phenolic and flavonoid compounds in the fruit extracts, which is evident in the peak current response of the cyclic voltammograms of the extracts (Figure 5).

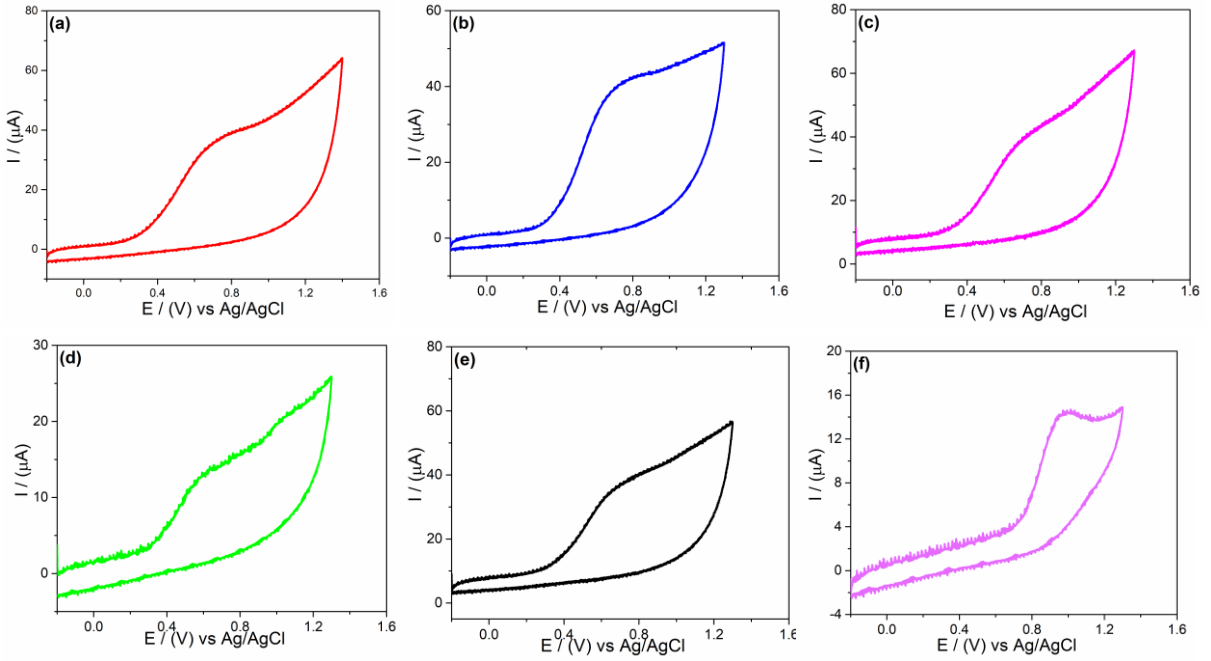

Figure 5. Cyclic voltammograms of methanolic extracts of various *Solanum aethiopicum* (0.17 g/mL): Ep1 (a), Ep2 (b), Ep3 (c), Ep4 (d), Ep5 (f), and Ep6 (f) recorded in 0.1 M phosphate buffer solution containing 0.1 M NaCl, pH 7.0, at a scan rate of 50 mV/s

CONCLUSION

This study assessed the total flavonoid and phenolic contents, as well as the antioxidant capacity, of six varieties of *S. aethiopicum L*. using spectroscopic and voltammetric methods. As observed, all six cultivars of *S. aethiopicum L* exhibited different flavonoid and phenolic levels in the extracts. Among the six cultivars of *S. aethiopicum*, EP6 and EP4 produced higher phenolic levels, measuring 146.74 µg GA-Eq/mg and 169.49 µg GA-Eq/mg, respectively. EP1 and EP6 produced the highest flavonoid contents of 222.20 µg Qc-Eq/mg and 178.67 µg Qc-Eq/mg, respectively. The bioactive compounds in the extract was found to be solvent-dependent; phenolics were more soluble in n-hexane, while flavonoids dissolved better in methanol. The antioxidant capacity increased with extract concentration across all six cultivars, indicating their potential as natural antioxidant sources. These findings reveal that *S. aethiopicum* is a valuable source of natural antioxidants with potential applications in functional foods and nutraceuticals. Future research should focus on exploring electrolytes that are effective at dissolving n-hexane extracts, isolating key bioactive compounds, and investigating *in vivo* therapeutic effects to improve understanding of their health applications.

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