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Spectrophotometric and voltammetric assessment of total flavonoid and phenolic content of *Cucurbita* species and their antioxidant activity

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Abstract

Evaluating the antioxidant properties of edible fruits is central to understanding their potential health benefits. We determined the antioxidant capacity and total phenolic and flavonoid contents of *Cucurbita pepo* and *Cucurbita moschata* using cyclic voltammetry and spectrophotometric assays, including 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP). A microwave-assisted method was used for extraction with *n*-hexane and methanol as solvents. The methanolic extracts produced higher phenolic contents in *C. pepo* (122.40 μg gallic acid equivalent (GA Eq)/mg) and *C. moschata* (132.4 μg GA Eq/mg) than the *n*-hexane extracts. Higher flavonoid levels were found in the methanolic extract of *C. pepo* (266.7 μg Q Eq/mg) and the *n*-hexane extract of *C. moschata* (177 μg Q Eq/mg). The DPPH assay showed higher percentage inhibition of free radicals in the *n*-hexane extracts of *C. pepo* (66.732 ± 6.976%) and *C. moschata* (58.425 ± 5.340%) than in the methanolic extracts. The FRAP assay indicated that the *n*-hexane extract of *C. pepo* had higher reducing power (~98.0%) than the methanolic extract (~77.5%), whereas the methanolic extract of *C. moschata* had higher reducing potential (~106.9%) than the *n*-hexane extract (~86.2%). The voltammetric assay showed that *C. moschata* had higher antioxidant activity (1.13 ± 0.061 μg GA Eq/mg and 4.19 ± 0.10 μg ascorbic acid equivalent (AA Eq)/mg) than *C. pepo* extracts (0.65 ± 0.004 μg GA Eq/mg and 3.40 ± 0.0068 μg AA Eq/mg). These findings provide insight into the antioxidant potential of two *Cucurbita* species and may support broader antioxidant research in food science and technology.

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1. Introduction

Cucurbitaceae is a large family of flowering plants comprising more than 130 genera and over 800 species [1]. The most popular genus in this family is pumpkin, also known as *Cucurbita*, with *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata* ranking among the most common species. Pumpkin is an economically important vegetable crop cultivated globally and can adapt to various environmental conditions. Most parts of pumpkin, including the peel, seeds, and flesh, are used as food, and they are rich sources of

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bioactive substances such as carotenoids, tocopherols, flavanols, polyphenolic compounds, vitamins, dietary fiber, and minerals [2–7]. These bioactive compounds are important for maintaining human health and preventing various diseases. For instance, phenols and flavonoids are known to exhibit good antioxidant properties, which play an important role in combating oxidative stress and protecting against food deterioration by neutralizing harmful free radicals [8]. Given the growing interest in natural antioxidants, which are safer than synthetic antioxidants that may pose a threat to human health, research is now directed toward identifying fruits and plants that are rich in phenolics and flavonoids.

The antioxidant capacity of food and plant extracts has mainly been investigated using spectrophotometric assays, such as ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assays [9–11]. Although these methods offer good sensitivity and high throughput, they are often limited by poor selectivity, matrix effects, poor physiological relevance, long analysis time, and results that are not always comparable across assays [9]. Because of these drawbacks, new methods for determining antioxidants are needed. Recently, electrochemical techniques have emerged as promising alternatives and complements to conventional methods for this purpose. The advantages of electrochemical methods include rapid analysis, high sensitivity, suitability for real-time or on-site analysis, minimal sample preparation, and applicability to coloured and turbid samples without interference [12, 13]. Despite these advantages, electrochemical methods also present limitations, such as electrode fouling and lack of selectivity. Therefore, combining spectroscopic and electrochemical methods can provide a complementary approach to determining antioxidants in plant and fruit extracts. Studies on the electrochemical determination of antioxidant capacity in different plant and fruit extracts have been reported [14–18].

Extraction is an important step for obtaining bioactive compounds with antioxidant properties from plant and fruit matrices. Conventional methods, such as maceration, solvent extraction, Soxhlet extraction, solid–liquid extraction, shaking, and vacuum extraction, have been widely used for decades. These traditional methods are simple and cost-effective. However, they have drawbacks, including high equipment costs, high solvent use, low extraction yields, prolonged extraction times, and the need for pure solvents, which limit their efficiency and sustainability [19]. In recent years, more sustainable extraction methods, such as ultrasound- and microwave-assisted extraction, have been employed to extract bioactive substances from plants, offering high efficiency, reduced solvent consumption, shorter extraction times, and improved product purity [20, 21]. Microwave-assisted extraction is becoming more popular because it relies on the thermal effects of microwave energy to heat the solvent and plant matrices, thereby enhancing the release of bioactive compounds. This technique offers many advantages, including low solvent use, high product yield and purity, rapid extraction, and environmental friendliness [22]. Reports are available on the use of microwave-assisted extraction for extracting bioactive compounds from plant materials with higher antioxidant activity than that obtained using traditional extraction methods [23–28].

Several factors, including cultivar, cultivation and storage environments, harvesting period, and extraction methods, affect the antioxidant properties of fruits and plants. Therefore, the antioxidant activity of *Cucurbita* needs to be assessed and compared with those of other plants. The lack of information on the antioxidant potential of *Cucurbita* species cultivated in this part of the country also motivated this work. Therefore, this study aimed to determine the antioxidant capacity and total flavonoid and phenolic contents of two *Cucurbita* species, namely *Cucurbita pepo* and *Cucurbita moschata* (Figure 1). Bioactive compounds were extracted using microwave-assisted extraction, and antioxidant capacity was evaluated using spectrophotometric and electrochemical assays.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used in the present work were of analytical grade.

2.2. Sample collection and preparation

The fruits of *C. pepo* and *C. moschata* were purchased from the old market in Wukari, Taraba State, Nigeria. Geographically, Wukari lies approximately between latitude 7°51' north and longitude 9°47' east of the Greenwich meridian. The fruit samples were washed, sliced into smaller pieces, and dried in the laboratory at room temperature. To increase the surface area, the dried samples were ground into powder using a commercial blender. The powdered samples were then stored in amber bottles until required for analysis.

2.3. Microwave-assisted extraction

The microwave-assisted extraction (MAE) procedure used to prepare the fruit extracts was adopted from previous studies [23, 24, 26, 29] using a conventional microwave oven (Model Daewoo, KOC-9Q4T).



Figure 1: Fruit samples of *Cucurbita pepo* (a) and *Cucurbita moschata* (b).

2.4. Determination of total phenolic content

The total phenolic content (TPC) of the extracts was evaluated using the Folin–Ciocalteu assay as reported previously [26, 30]. Briefly, 0.1 mL of extract was added to 0.1 mL of Folin–Ciocalteu reagent in a 10 mL volumetric flask, and distilled water was added to make a final volume of 10 mL. Distilled water was used as the blank reagent. 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 allowed to stand for 20 min. The sample absorbance was measured at 760 nm using a UV–visible spectrophotometer (Multiskan GO 1.00.40). The phenolic content in the extract was expressed as μg of gallic acid equivalent (GA Eq) per mg of dry sample using a standard calibration curve established previously.

2.5. Determination of total flavonoid content

The total flavonoid content (TFC) in the extracts was measured using previous methods [26, 31, 32]. Briefly, approximately 40 μL of extract was diluted with 1.25 mL of distilled water and mixed with 15 μL of $NaNO_2$. After 5 min, 30 μL of $AlCl_3$ was added, and the mixture was incubated for 10 min. Subsequently, 50 μL of 1 M NaOH was added and thoroughly mixed. Absorbance was measured immediately at 510 nm using a UV–visible spectrophotometer (Multiskan GO 1.00.40). The flavonoid content in the fruit extracts was expressed as μg of quercetin equivalent (Q Eq) per mg of dry sample using a standard calibration curve established previously.

2.6. Evaluation of antioxidant capacity

The antioxidant capacity of the fruit samples was assessed using DPPH, FRAP, and cyclic voltammetry assays, as described below. All measurements were performed in triplicate.

2.6.1. DPPH scavenging assay

The DPPH assay was performed using a previous procedure [33, 34]. Briefly, a 0.3 mM solution of DPPH was prepared in methanol, and 1 mL of the resulting solution was reacted with 1 mL of fruit extract sample and 1 mL of methanol in a volumetric flask. The mixture was incubated for 30 min, after which the absorbance of the sample was recorded at 517 nm using a UV–visible spectrophotometer (Multiskan GO 1.00.40). The antioxidant capacity of the fruit extracts was expressed as percentage inhibition using Eq. (1):

$$\%\ \text{inhibition} = \left(\frac{A_C - A_S^*}{A_C}\right) \times 100, \tag{1}$$

where $A_S^* = A_S - A_B$, A_S is the absorbance of the fruit extract with DPPH, A_B is the absorbance of the fruit extract only, and A_C is the absorbance of DPPH only.

The effective amount of fruit extract required to scavenge 50% of DPPH free radicals, commonly referred to as IC_{50} (mg/mL), was determined by interpolating concentration versus absorbance data from linear regression analysis using Eq. (2):

$$IC_{50} = C_1 + \left(\frac{50 - I_1}{I_2 - I_1}\right) \times (C_2 - C_1), \tag{2}$$

where IC_{50} is the concentration of the fruit extract required to inhibit the oxidation process by 50%, C_1 is the concentration resulting in inhibition just below 50% (I_1), C_2 is the concentration resulting in inhibition just above 50% (I_2), I_1 is the percentage inhibition observed at concentration C_1 ($I_1 < 50$), and I_2 is the percentage inhibition observed at concentration C_2 ($I_2 > 50$).

2.6.2. Ferric reducing antioxidant potential assay

The FRAP assay was performed as reported in the literature [35, 36]. Briefly, different concentrations of the extract 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 and incubated at 50 °C for 20 min. Then, 0.5 mL of trichloroacetic acid was added. A 2 mL aliquot of the solution was mixed with 2 mL of distilled water and 0.2 mL of $FeCl_3{\cdot}6H_2O$. The mixture was allowed to stand for 10 min for colour development, and absorbance was measured at 700 nm using a UV–visible spectrophotometer (Multiskan GO 1.00.40). The reducing power of the fruit extracts was determined using Eq. (3):

$$\%\,\text{FRAP} = \left(\frac{A_a - A_b}{A_a}\right) \times 100, \tag{3}$$

where A_a is the absorbance of the fruit extract and A_b is the absorbance of the blank.

During UV–visible analysis, a disposable microplate in a microplate reader was used rather than a standard cuvette; therefore, cleaning or sterilization was not required.

2.6.3. Cyclic voltammetric assay

Cyclic voltammetric measurements were performed using an open-source portable potentiostat (RodeoStat, RSTAT-01, USA) controlled with Rodeostat Web App V1.2.1 software. The experimental configuration consisted of a three-electrode system with a commercial glassy carbon disk as the working electrode (3 mm diameter), a platinum wire counter electrode, and Ag/AgCl (3 M KCl) as the reference electrode. Before each measurement, the glassy carbon electrode was polished using a slurry of 0.05 μm alumina powder. The potential of the working electrode was scanned between 0 and +1.3 V vs. Ag/AgCl at 50 mV/s. The electrochemical cell and electrodes were thoroughly cleaned with distilled water, rinsed with a small volume of acetone, and dried after each sample measurement to avoid sample contamination. The antioxidant capacity of the *Cucurbita* fruit extracts was calculated from standard calibration plots of ascorbic and gallic acids using Eq. (4) [30, 37, 38]:

$$\text{AOC}\,(\mu\text{g/mg}) = \frac{C_{\text{eq}}}{C}, \tag{4}$$

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

3. Results and discussion

3.1. Total phenolic and flavonoid contents of fruit extracts

Phenolics and flavonoids are among the known bioactive substances with important medicinal applications owing to their antioxidant and anti-inflammatory properties [7]; therefore, their contents in the fruit extracts were first evaluated. The phenolic content in the extracts was expressed as gallic acid equivalent using the calibration plot equation $y = 0.00331x + 0.0719$ (Figure A1). Table 1 presents the phenolic contents measured in the fruit samples. Phenolic content was higher in the methanolic extract of *C. moschata* (132.4 μg GAE/mg) and *C. pepo* (122.440 μg GAE/mg) than in the *n*-hexane extracts (116.090 μg GAE/mg in *C. moschata* and 0 μg GAE/mg in *C. pepo*). The results indicate that the extraction ability for phenolics is likely to depend on solvent polarity; methanol, being more polar, can efficiently extract phenolic compounds, whereas *n*-hexane, a non-polar solvent, is suitable for extracting lipophilic bioactive compounds. Liu *et al.* [39] observed that the polarities, solubilities, and chemical structures of solvents used during extraction play crucial roles in extracting phenolic compounds.

The flavonoid level in the two *Cucurbita* extracts was expressed as quercetin equivalent from the calibration plot equation $y = 0.0005x + 0.10183$ (Figure A2). Table 1 also presents the total flavonoid contents of the *C. pepo* and *C. moschata* extracts. The methanolic extract of *C. pepo* had higher flavonoid content (266.733 μg Q Eq/mg) than the *n*-hexane extract (80.400 μg Q Eq/mg). By contrast, the *n*-hexane extract of *C. moschata* showed higher flavonoid content (177.000 μg Q Eq/mg) than the methanolic extract (126.400 μg Q Eq/mg). These results reveal notable differences in flavonoid contents. The experimental differences in flavonoid content can be attributed to several factors, including pumpkin maturity, varieties, soil types, and geographical locations of the two *Cucurbita* species [40–42]. Both *n*-hexane and methanol demonstrated efficient extraction power for phenolic and flavonoid compounds. The flavonoid contents obtained in this study are higher than those reported previously for *C. moschata* extracts [43].

Table 1: TPC and TFC values of the fruit extracts of *Cucurbita pepo* and *Cucurbita moschata*.

Extract	TPC (μg GAE/mg)		TFC (μg Q Eq/mg)	
	C. pepo	*C. moschata*	*C. pepo*	*C. moschata*
n-Hexane	0.00	116.09	80.40	177.00
Methanol	122.44	132.40	266.73	126.40

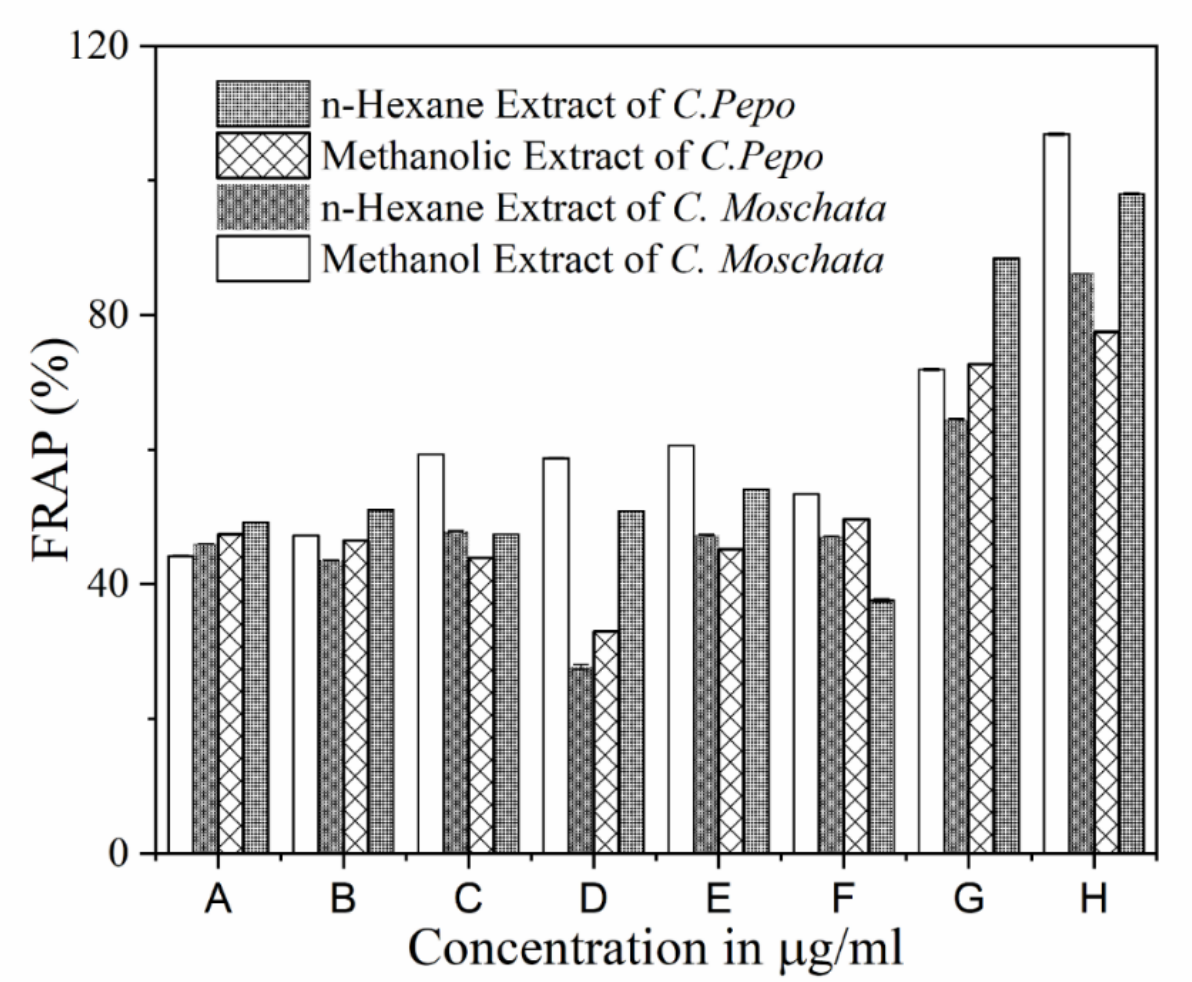


Figure 2: Reducing power of *n*-hexane and methanolic extracts of *C. pepo* and *C. moschata* (A = 7.8125 μg/mL; B = 15.625 μg/mL; C = 31.25 μg/mL; D = 62.50 μg/mL; E = 125.00 μg/mL; F = 250 μg/mL; G = 500.00 μg/mL; H = 1000.00 μg/mL).

3.2. Evaluation of antioxidant activity

3.2.1. FRAP assay

The FRAP assay is one of the most versatile methods for measuring the antioxidant properties of bioactive substances. The method is based on the tendency of bioactive compounds to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by forming a blue complex [44]. In other words, the FRAP assay assesses antioxidant capacity through electron donation to reduce ferric ions. Figure 2 depicts the reducing power determined for the *n*-hexane and methanolic extracts of *C. pepo* and *C. moschata*. The two *Cucurbita* species displayed good antioxidant capacity, with the methanolic extract of *C. moschata* showing the highest activity in the analysis. At the same concentration of 1000 μg/mL, the *n*-hexane extract of *C. pepo* exhibited higher reducing activity (~98.0%) than the methanol extract (~77.5%). By contrast, the methanolic extract of *C. moschata* had higher reducing power (~106.9%) than the *n*-hexane extract (~86.2%) at a similar concentration of 1000 μg/mL of the fruit extracts. The results indicate that both *n*-hexane and methanolic extracts have a significant tendency to reduce ferric ions as extract concentration increases and can therefore act as potential antioxidants.

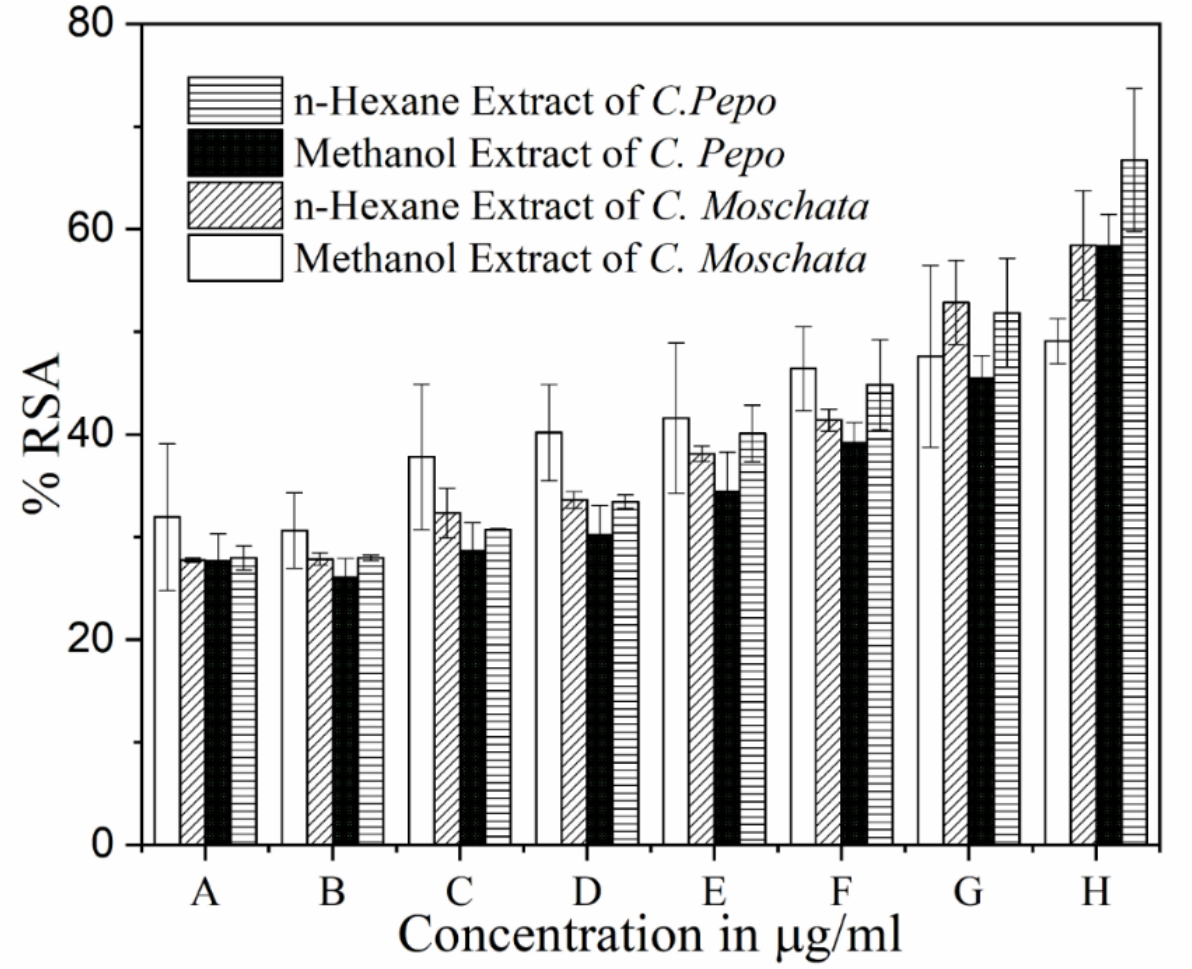


Figure 3: Variation in DPPH percentage inhibition of *n*-hexane and methanolic extracts of *C. pepo* and *C. moschata* (A = 7.8125 μg/mL; B = 15.625 μg/mL; C = 31.25 μg/mL; D = 62.50 μg/mL; E = 125.00 μg/mL; F = 250 μg/mL; G = 500.00 μg/mL; H = 1000.00 μg/mL).

3.2.2. DPPH scavenging assay

DPPH is widely used to determine antioxidant activity. The method is particularly sensitive to compounds that can act as hydrogen or electron donors and neutralize DPPH free radicals through a colour change from purple to yellow because of the formation of diphenylpicrylhydrazine. Antioxidant capacity was recorded based on the scavenging activity of the stable DPPH free radical, and the results, expressed as percentage inhibition, are shown in Figure 3. The percentage inhibition of DPPH free radicals by the fruit extracts was concentration-dependent. The DPPH test showed that the *n*-hexane extract of *C. pepo* had higher antioxidant activity (66.73 ± 6.98%) than the methanolic extract, which had 58.37 ± 3.05% inhibition at the same concentration of 1000 μg/mL. Both *n*-hexane and methanolic extracts of *C. pepo* exhibited similar antioxidant activity, with 27.95 ± 0.28% and 27.67 ± 2.64% inhibition, respectively, at 15.625 μg/mL. At 1000 μg/mL, the antioxidant activity of *C. moschata* was higher in the *n*-hexane extract (58.43 ± 5.34%) than in the methanolic extract (49.07 ± 2.21%). These findings align with a previous study [7]. Dar *et al.* [45] investigated the role of several solvents on the antioxidant potential of *Cucurbita pepo* L. leaf extracts. They observed that ethyl acetate (79.44%), *n*-butanol (68.9%), and aqueous (59.96%) extracts had strong DPPH radical inhibition activity, whereas the lowest antiradical properties were found for chloroform (47.56%) and *n*-hexane extracts (40.5%).

The antioxidant potentials of the fruit extracts were further expressed in terms of IC_{50}. The IC_{50} indicates the antioxidant concentration required to reduce 50% of the free radical concentration, and this value is inversely proportional to the antioxidant capacity of compounds. A lower IC_{50} value denotes higher antioxidant potential of the bioactive compound, and vice versa [46]. The IC_{50} results further show that the *n*-hexane extracts of *C. pepo* (IC_{50} = 435.371 μg/mL) and *C. moschata* (IC_{50} = 437.832 μg/mL) had better antioxidant capacity than the methanolic extract of *C. pepo* (IC_{50} = 675.800 μg/mL).

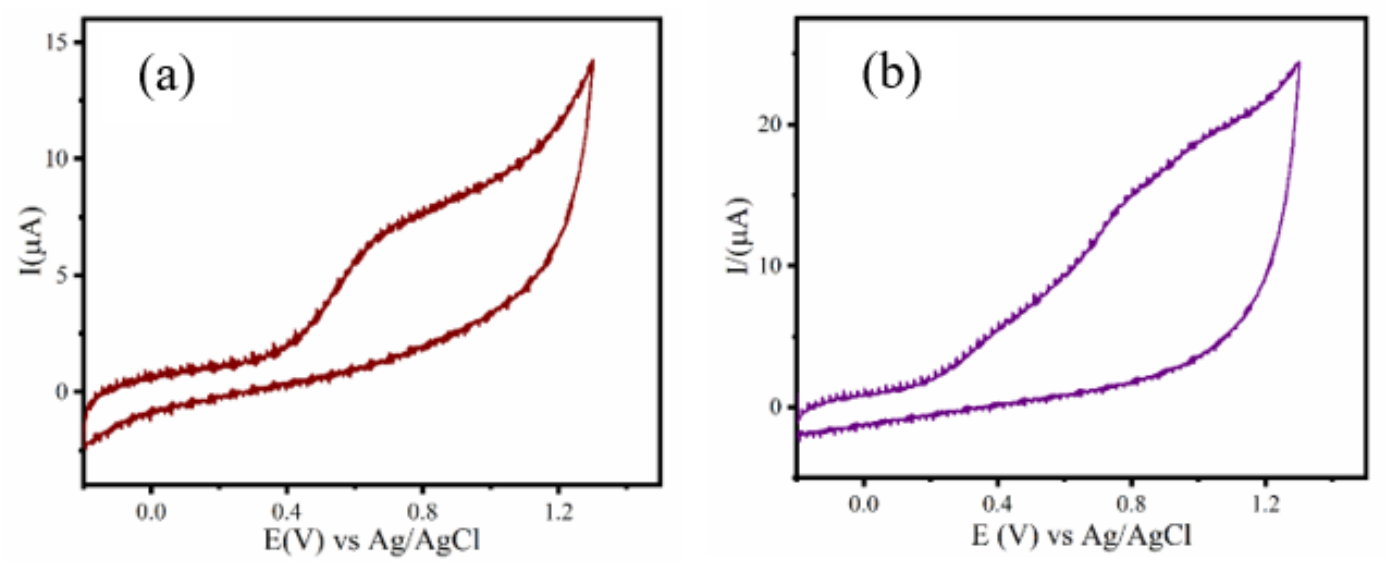


Figure 4: Cyclic voltammograms of methanolic extracts of (a) *C. pepo* and (b) *C. moschata*, recorded in 0.1 M phosphate buffer solution, pH 7.4, at a scan rate of 50 mV/s.

Table 2: Antioxidant values of the methanolic extracts of *C. pepo* and *C. moschata* determined using the cyclic voltammetry assay.

Extract	Peak potential, E_p (V)	Antioxidant capacity	
		μg GA Eq/mg	μg AA Eq/mg
C. pepo	0.79 ± 0.02	0.65 ± 0.01	3.40 ± 0.01
C. moschata	1.07 ± 0.08	1.13 ± 0.06	4.19 ± 0.10

3.2.3. Electrochemical assay

The electrochemical method was used to measure the antioxidant capacity of the fruit extracts. Figure 4 depicts the cyclic voltammograms recorded from the methanolic extracts of *C. pepo* (Figure 4a) and *C. moschata* (Figure 4b) with a bare glassy carbon electrode (GCE) at a sweep rate of 50 mV/s. A clear oxidation peak was observed, with peak potentials of ~0.79 V and 1.072 V vs. Ag/AgCl for the *C. pepo* and *C. moschata* extracts, respectively. The difference in peak potential may indicate the presence of different bioactive compounds [47].

The calculated antioxidant capacities of the methanolic extracts of *C. pepo* and *C. moschata* are shown in Table 2. The fruit extract of *C. moschata* showed higher antioxidant activity (1.13 ± 0.061 μg GA Eq/mg and 4.19 ± 0.10 μg AA Eq/mg) than the *C. pepo* extract (0.65 ± 0.004 μg GA Eq/mg and 3.40 ± 0.0068 μg AA Eq/mg). The content and activity of redox-active antioxidant compounds in the extracts are directly linked to the peak current responses. *C. moschata* appears to have a superior antioxidant profile in terms of both phenolic content (GAE) and ascorbic acid-like activity (AAE), as evidenced by the noticeably higher phenolic content values observed in this plant extract. Conversely, the comparatively lower antioxidant values of *C. pepo* suggest a less prominent presence of these bioactive compounds. This difference could stem from specific variations in the phytochemical content of the fruit extracts. The higher antioxidant capacity of *C. moschata* indicates greater potential for scavenging free radicals and providing oxidative protection.

Therefore, *C. moschata* is a more promising candidate for nutraceutical and functional food applications, especially those targeting antioxidant enrichment. Although *C. pepo* also exhibits antioxidant potential, its lower electrochemical response points to reduced antioxidant efficacy, which may limit its use in applications requiring strong antioxidant potency.

4. Conclusion

This study assessed the antioxidant activity and total phenolic and flavonoid contents of two *Cucurbita* species, *Cucurbita pepo* and *Cucurbita moschata*, using cyclic voltammetric and UV–visible spectrophotometric assays. Microwave-assisted extraction proved to be a faster and greener extraction method. Both methanol and *n*-hexane demonstrated good extraction power for the bioactive compounds. The findings revealed that the *Cucurbita* species studied are rich sources of phenolic and flavonoid compounds. Combining multiple analytical techniques can provide a powerful basis for determining the antioxidant properties of fruits and offers a robust model for future research on plant-based antioxidants.

Data availability

The data obtained during the process of this research work are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no competing financial interests.

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Appendix

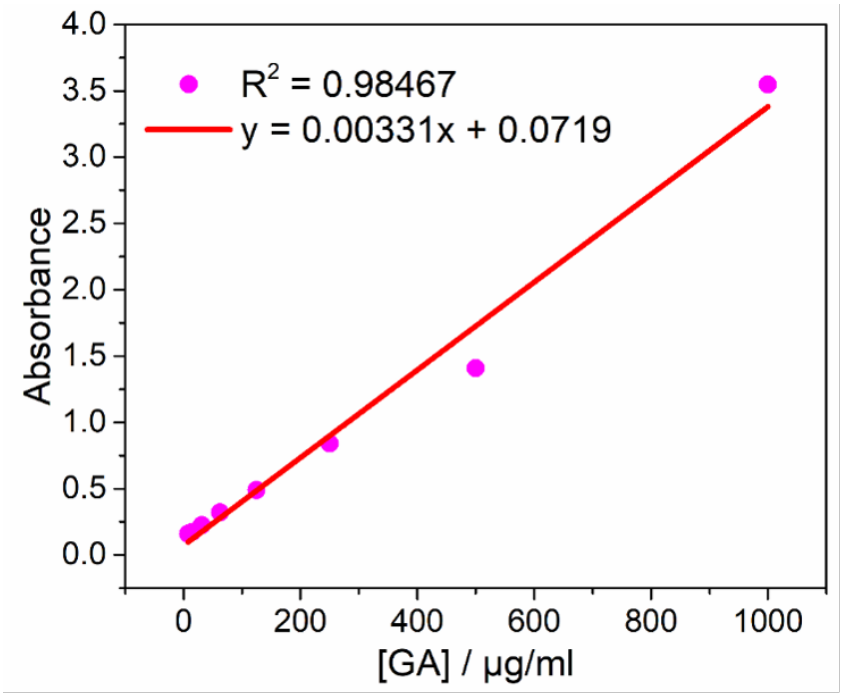


Figure A1: Calibration plot of gallic acid used for determination of total phenolic content.

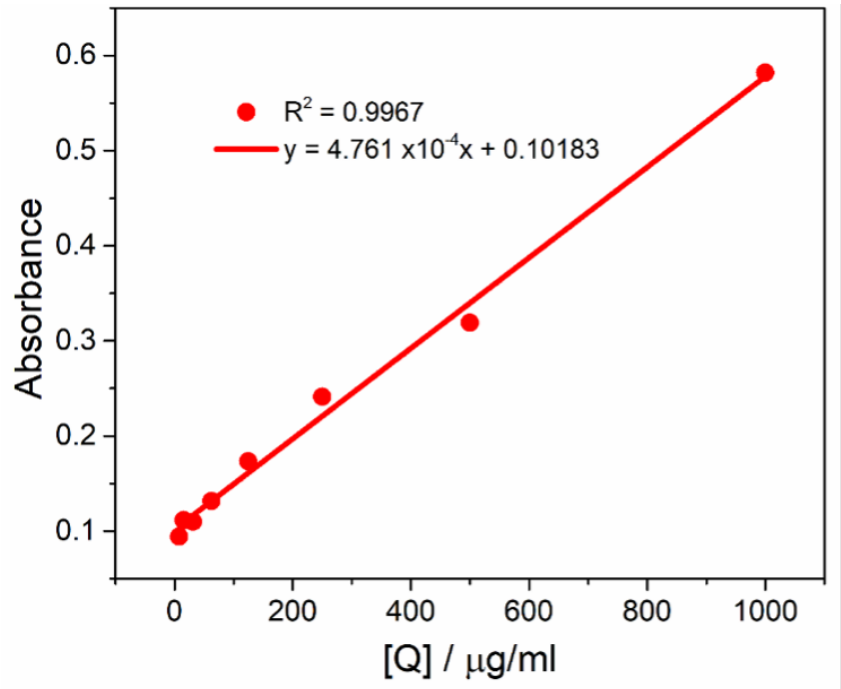


Figure A2: Calibration plot of quercetin used for determination of total flavonoid content.