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Evaluation of Antioxidant Capacity of *Momordica charantia* and *Trichosanthes cucumerina* Using Electrochemical and Spectrophotometric Methods

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ABSTRACT

Antioxidants are compounds that inhibit oxidation at low concentrations and are beneficial to human health. In this study, the antioxidant capacity of two Cucurbitaceae family members, *Momordica charantia* and *Trichosanthes cucumerina* , was evaluated using spectrophotometric methods (DPPH radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP)) and electrochemical method (cyclic voltammetry). The fruit extracts were prepared with methanol and hexane using microwave-assisted extraction. The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were also determined. Hexane extracts of both species had higher phenolic content than methanolic extracts. For flavonoids, *M. charantia* hexane extract showed the highest value (265.80 µg QE/mg) compared to its methanol extract (85.27 µg Q Eq/mg), whereas *T. cucumerina* methanol extract (122.27 µg Q Eq/mg) was higher than its hexane extract (76.47 µg QE/mg). The DPPH assay showed stronger radical scavenging in *T. cucumerina* (IC_{50} = 214.80 µg/mL) than *M. charantia* (IC_{50} = 402.86 µg/mL). In FRAP, reducing power increased with concentration, with hexane extracts showing higher activity (163.00% for *T. cucumerina*, 103.00% for *M. charantia*) than methanol extracts. Cyclic voltammetry revealed *T. cucumerina* had greater antioxidant capacity than *M. charantia*. Overall, *T. cucumerina* demonstrated stronger antioxidant potential, highlighting its value as a natural antioxidant source.

Keywords: Antioxidants, cyclic voltammetry, microwave-assisted extraction (MAE), spectroscopy, *Momordica charantia*, *Trichosanthes cucumerina.*

INTRODUCTION

An antioxidant inhibits or halts the oxidation of a substrate at low concentrations. It refers to a molecular entity that, at relatively low levels, inhibits, delays, or prevents the oxidation of a vulnerable substrate by capturing free radicals, forming complexes with pro-oxidant metals, or helping to decompose reactive species that stem from oxygen and nitrogen. Most antioxidants

come from natural or synthetic sources, depending on whether they are made in a laboratory or produced by living organisms. They include commonly eaten foods such as oranges, watermelons, apples, grapes, turmeric, ginger, pears, melons, cashews, spinach, swiss chard, cabbage, and onions. The antioxidants are further classified into smaller groups based on their size (small and large molecules), solubility (fat-soluble and water-soluble), and bioactivity (enzymatic and non-enzymatic). Natural antioxidants have shown strong antibacterial activity, potentially making them a good alternative to synthetic ones [1-6]

Antioxidants are added to foods to prevent degradation caused by free radicals and to stop oxidative changes. Lipid rancidity can be avoided by using antioxidants as stabilisers [4,7]. A study found that the antioxidant capacity of polyphenols in rosemary flowers (*Rosmarinus officinalis* L.) can also be used for anti-ageing benefits [8], as shown in *Caenorhabditis elegans* [9]. Various compounds and extracts from plants, grains, and fruits are used as natural antioxidants in the cosmetics industry. These substances can protect products from oxidative damage or reduce oxidative stress on the skin [4].

Traditional extraction techniques such as maceration, sonication, percolation, turbo-extraction, and Soxhlet extraction have been used, but they have limitations, including long processing times, the need for large amounts of solvents, and unsuitability for heat-sensitive compounds [10-12]. MAE is used because of its advantages, such as speed, efficiency, and high yield of bioactive compounds [13-18]. As a result, identifying and measuring antioxidants from plants is a key focus in food, medicine, and biology.

Phenolic and flavonoid molecules are among the most studied plant chemicals because of their strong antioxidant abilities. The antioxidant activity of plant extracts has often been tested with spectrophotometric methods like FRAP and DPPH [19-21]. However, there are concerns about measuring antioxidant activity in turbid extracts of fruits, vegetables, or herbs using these methods.

Electrochemical techniques such as voltammetry offer benefits like direct testing, quick results, and high sensitivity [22]. This easy and cost-effective electrochemical method has become a good alternative to spectrophotometric tests for assessing the antioxidant capacity of plant samples. Techniques like cyclic voltammetry and differential pulse voltammetry have been used in research on the antioxidant potential of various plants [22-27].

Several bioactive substances, including flavonoids and phenols, are found in *Momordica charantia* and *Trichosanthes cucumerina*. *Momordica charantia*, a well-known medicinal plant in the Cucurbitaceae family, is often called bitter gourd or bitter lemon. It is rich in antioxidants and is believed to have anticancer, antidiabetic, antiulcer, antitumor,

antimicrobial, cytotoxic, and antiviral effects. Traditionally, bitter gourd has been used to treat conditions such as toothache, diarrhoea, boils, diabetes, dysmenorrhoea, eczema, emmenagogue, galactagogue, gout, jaundice, kidney stones, leprosy, leucorrhoea, piles, pneumonia, psoriasis, rheumatism, hepatitis, and scabies [28-31]. Similarly, *Trichosanthes cucumerina*, another important member of the Cucurbitaceae family, bears fruit up to one metre long. The whole plant, including roots, leaves, fruits, and seeds, shows antidiabetic, antibacterial, anti-inflammatory, anthelmintic, febrifuge, gastroprotective, and antioxidant properties. Its potent antioxidant activity may help prevent chronic diseases such as heart illness. It is also traditionally used across cultures to treat fever, headaches, and stomach issues, demonstrating its versatility [32-37].

To date, only studies employing spectroscopic methods have been reported measuring the antioxidant capacity of *Momordica charantia* and *Trichosanthes cucumerina*. [28,33-34,39]. Therefore, this study employed both spectrophotometric including (DPPH, FRAP) and cyclic voltammetry methods to determine the Antioxidant Capacity (AOC) of *Momordica charantia* and *Trichosanthes cucumerina* (Plate 1).

Plate 1. Fruit sample of (a) *Momordica charantia* and (b) *Trichosanthes cucumerina*

Chemicals

The chemicals used in this study were of analytical grade. Sodium chloride (BDH Chemicals LTD., 99.5%), Folin-Ciocalteu (Sigma-Aldrich), 2,2'-diphenyl-1-picrylhydrazyl (DPPH, Selleck Chemicals, 99.05%), quercetin (Nutri Avenue, 98%), ascorbic acid (Cowin Industry, 99%), gallic acid (Sigma-Aldrich, >97.5%), sulphuric acid (Merck, 98%), sodium dihydrogen

phosphate (NaH_2PO_4, 98%, Loba Chemie PVT LTD), disodium hydrogen phosphate (Na_2HPO_4, 98.5%, Molychem), TPTZ (2,4,6-tripyridyl-s-triazine, Sigma-Aldrich, 98%), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, Merck, 98%), sodium hydroxide (NaOH, Merck, 98%), methanol (Sigma-Aldrich, 99.9%, HPLC grade), n-hexane (Fisher Scientific, 95%), aluminium chloride ($AlCl_3$, Gujarat Alkalies and Chemicals Limited via PCAPL India, 99.3%), and sodium carbonate (Na_2CO_3, Guangzhou Sunray Import and Export CO., LTD., 99.2%)

Microwave-Assisted Extraction of Fruit Extracts

Microwave-assisted extraction of *Momordica charantia* and *Trichosanthes cucumerina* extracts was carried out by adopting the method suggested by Habila et al [40] with slight modifications using a domestic microwave system (Daewoo, KOC-9Q4T). Approximately 120 g of powdered fruit from *Momordica charantia* and *Trichosanthes cucumerina* was weighed into a separate Bama bottle, and 200 mL of n-hexane was added to each bottle and allowed to stand for around 30 minutes. The bottle was tightly covered, placed into the microwave oven, irradiated at a temperature of 150 °C on defrost mode, and microwaved five times each at 3-minute pulses, then removed and allowed to cool in-between pulses. The plant extract was washed with n-hexane and filtered using a muslin cloth. This procedure was repeated with a methanol/water mixture in a 70:30 ratio. The extracts obtained were concentrated using a rotary evaporator at 40 °C and subsequently dried at room temperature.

Determination of Total Phenolic Content

The total phenolic content (TPC) of the *Momordica charantia* and *Trichosanthes cucumerina* extracts was determined according to the Folin-Ciocalteu method [40]. Briefly, 0.1 mL of the extract was added to a 0.1 mL Folin-Ciocalteu reagent in a volumetric flask, and 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 using a UV-spectrophotometer (Multiskan GO 1.00.40). The total phenolic content of the sample was expressed as µg of gallic acid equivalent per mg of sample (µg GA Eq/mg) using the calibration curve.

Determination of Flavonoid Content

Momordica charantia and *Trichosanthes cucumerina* extracts (40 µL) were diluted with 1.25 mL of distilled water and mixed with 15 mL of $NaNO_2$. After 5 minutes, 30 µL of $AlCl_3$ was added, and the mixture was then incubated for an additional 10 minutes. Subsequently, 50 µL of 1 M NaOH was added. The absorbance was measured immediately at 510 nm using a UV-spectrophotometer (Multiskan GO 1.00.40). The Total flavonoid content (TFC) of the extract samples of *Momordica charantia* and *Trichosanthes cucumerina* was expressed as µg of Quercetin equivalent per mg of extract (µg Q Eq/mg) from the calibration curve [42-43].

Antioxidant Capacity Measurement by Spectrophotometric Method

DPPH radical scavenging activity

Antioxidant activity was assessed using DPPH, a stable free radical commonly employed to evaluate the radical scavenging ability of antioxidant compounds. This technique relies on the reduction of DPPH in a methanol solution when exposed to a hydrogen-donating antioxidant, resulting in the formation of the non-radical DPPH-H [44]. This reduction causes a colour change from purple to yellow, which is measured spectrophotometrically at 517 nm. The reaction mixture (3.0 mL) contains 1.0 mL of DPPH in methanol (0.3 mM), 1.0 mL of the extract, and 1.0 mL of methanol. The percentage inhibition is calculated using equation (1) [45-46]. The effective concentration of the sample needed to scavenge 50% of DPPH radicals (IC_{50} mg/ml) is determined by interpolating concentration versus absorbance data from a linear regression analysis.

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

AS* = AS − AB, AS = Absorbance of the sample + DPPH, AB = Absorbance of the sample only

AC = Absorbance of DPPH only

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

C_1 and C_2 two concentration, I_1 and I_2 Inhibition %

Ferric Reducing Antioxidant Potential

Different series of extract concentrations 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 trichloroacetic acid was added. 2 mL of the solution was mixed with 2 mL of distilled water and 0.2 mL of $FeCl_3 \cdot 6H_2O$. This was allowed to stand for 10 minutes for colour development,

and absorbance was measured at 700 nm using a spectrophotometer (Multiskan GO 1.00.40). The reducing power of the *Momordica charantia* and *Trichosanthes cucumerina* extracts was expressed using equation 3 [47-48]:

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

A_a: Absorbance of the extract, A_b: Absorbance of the blank

Antioxidant Capacity Evaluation by Cyclic Voltammetric Method

The voltammetric experiment was conducted using the cyclic voltammetric method in a 5 mL glass electrochemical cell with three electrodes: a glassy carbon disk (working electrode, 3 mm in diameter), a platinum wire as the counter electrode, and an Ag/AgCl (saturated KCl) 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 gadget potentiostat (Rodeostat, RSTAT-01, USA). The antioxidant capacity of the *Momordica charantia and Trichosanthes cucumerina* fruit extract samples was obtained from calibration curves using equation 4 with ascorbic and gallic acids as standards [24, 41-42]

$$AOC\ (g/mg) = \frac{C_{eq}}{C} \quad (4)$$

where AOC 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 *Momordica charantia* and *Trichosanthes cucumerina* using spectrophotometric and cyclic voltammetric methods. All n-hexane extracts resulted in an oily product, while methanolic extracts were in powdered form.

Percentage Yield of the Extracts

The percentage yields (% w/w) of extracts from the fruits of *Momordica charantia* and *Trichosanthes cucumerina* are presented in Table 1.

Table 1. Percentage yield of the extracts

Extracts	Percentage Yield	
	Trichosanthe cucumerina	*Momordica charantia*
Hexane extract	1.33	10.08
Methanol extract	1.67	12.00

Results indicate that methanolic extracts relatively show higher yield compared to the n-hexane extracts. This difference in the percentage yields might be attributed to the difference in the polarities of the solvents, which also play a key role in increasing the solubility of phytochemical compounds [49]. The polarity effect of the solvents justifies this result, and similar findings have been reported in the literature [24]. Recent studies have also shown that the method used for the extraction of bioactive compounds can influence the yield of the extracts [34].

Total Phenolic Content and Total Flavonoid Content

Table 2 shows the results for the phenolic and flavonoid contents in the extracts of *Trichosanthes cucumerina* and *Momordica charantia*.

Table 2: Total Phenolic Content and Total Flavonoid Content of the Fruit Extracts of *Trichosanthe cucumerina and Momordica Charantia*

Extracts	TPC (µg GA Eq/mg)		TFC (µg Q Eq/mg)	
	Trichosanthe cucumerina	*Momordica charantia*	*Trichosanthe cucumerina*	*Momordica charantia*
Hexane extract	113.636	149.11	76.467	265.80
Methanol extract	88.465	105.61	122.267	85.27

The phenolic and flavonoid contents show variations in the extracts. Table 2 reveals that the hexane extract of *Momordica charantia* exhibits higher flavonoid content (265 80 µg Q Eq./mg) than the methanolic extract (85.27 µg Q Eq./mg). On the other hand, the methanolic extract recorded higher flavonoid content in the extract of *Trichosanthes cucumerina* (122.267 µg Q Eq./mg) compared to the hexane extract (76.467 µg Q Eq./mg).

The phenolic content in the extract was found to be higher in both the hexane extracts of *Trichosanthes cucumerina* (113.636 µg GA Eq/mg) and *Momordica charantia* (149.11 µg GA Eq/mg) than in the methanol extracts of the same fruits. The higher values of the Total

Phenolic Content (TPC) in the hexane extract compared to the methanolic extract could be attributed to the use of the microwave-assisted extraction method, which has been reported recently to influence the phenolic yields of an extract when compared to the conventional Soxhlet extraction method [34]. The oily extract by microwave-assisted extraction obtained in this study showed higher TPC and better antioxidant properties than the oil extracted with the conventional Soxhlet technique [34]. Previous studies have also demonstrated that the method applied for extraction influences the final properties of the extracts [50-51]. The TFC and TPC found in this study are comparatively higher than those reported previously [39], which could be attributed to differences in solvent polarity and method of extraction.

FRAP Assay of *Trichosanthe cucumerina* and *Momordica charantia*

Figure 1 presents the result of reducing power of *T. cucumerina* and *M. charantia* evaluated through the FRAP assay.

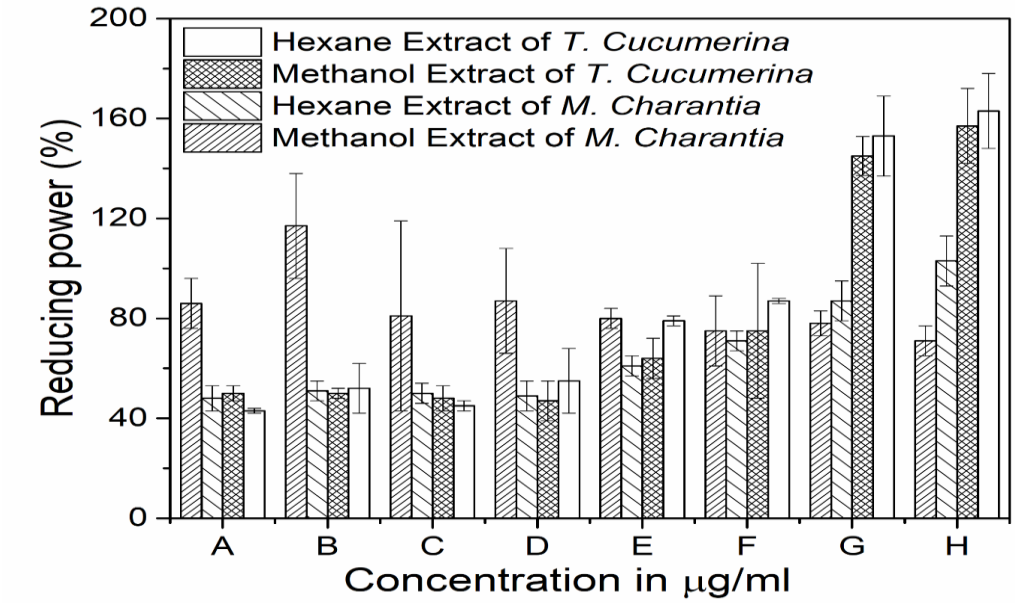

Figure 1. Reducing power of hexane and methanolic extracts of *Trichosanthe cucumerina* and *Momordica charantia* (A=7.8125 µg/ml; B=15.625 µg/ml; C=31.25 µg/ml; D=62.50 µg/ml; E=125.00 µg/ml; F=250 µg/ml; G=500.00 µg/ml; H=1000.00 µg/ml)

The results indicate that the reducing power of the extract increases with an increase in the extract concentration. The hexane extract of *T. cucumerina* and *M. charantia* exhibits higher percentage of reducing ability at the maximum concentration of 1000 µg/ml (~163.00% for *T. cucumerina* and ~103.00% for *M. charantia*) compared to the methanolic extracts (~157.00% for *T. cucumerina* and ~71.00% for *M. charantia*). This indicates that the hexane extract has a

stronger reducing activity for both fruit extracts. Similar trend was observed in a previous study where the oil of *Curcuma longa L.* extracted through microwave-assisted extraction showed better antioxidant properties than oil from Soxhlet extraction [34]. Hence, this has shown that the microwave-assisted extraction method influenced the final properties of the extracts of *T. cucumerina* and *M. charantia*.

DPPH Assay of *Trichosanthe cucumerina* and *Momordica charantia*

DPPH assay results of the fruit extracts of *Trichosanthes cucumerina* and *Momordica charantia* are shown in Figure 2.

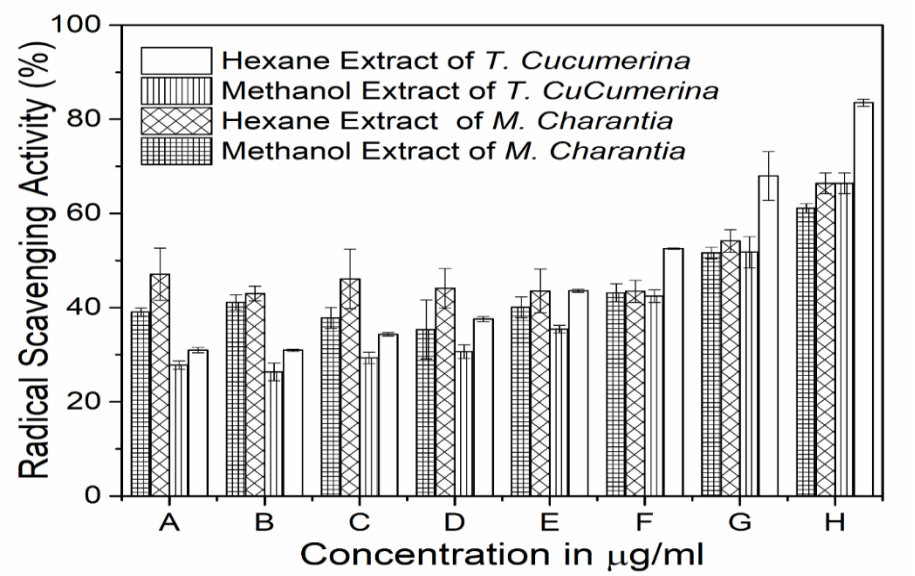

Figure 2. Variation of the percentage inhibition of DPPH of hexane and methanolic extracts of *Trichosanthe cucumerina* and *Momordica charantia* (A=7.8125 µg/ml; B=15.625 µg/ml; C=31.25 µg/ml; D=62.50 µg/ml; E=125.00 µg/ml; F=250 µg/ml; G=500.00 µg/ml; H=1000.00 µg/ml)

The radical scavenging activity (RSA) of the fruit extracts showed significant differences across the two extracts, with hexane extracts displaying high RSA values (*Trichosanthes cucumerina:* 83.50±0.75%; *Momordica charantia:* 66.39±2.21%), compared to the methanolic extracts (*Trichosanthes cucumerina:* 66.39 ±2.21%; *Momordica charantia:* 61.11±0.99%) at the highest concentration of 1000 µg/ml. The DPPH radical scavenging activity found in the present study is higher than that reported in the literature for *Momordica charantia* fruits [28]. Furthermore, the IC_{50} results calculated shows that the hexane extracts of *Trichosanthes cucumerina* (214.832 µg/ml) and *Momordica charantia* (403.575 µg/ml) have the lower values, which indicates higher activity in the n-hexane extract as compared with the higher IC_{50}

values in the methanolic extracts of *Trichosanthes cucumerina* (452.579 µg/ml) and *Momordica charantia* (452.550 µg/ml). These findings suggest that the hexane extract of *Trichosanthes cucumerina* has the higher radical scavenging activity.

Evaluation of Antioxidant Capacity by Cyclic Voltammetry Method

The antioxidant capacity of the methanolic extracts of *Momordica charantia* and *Trichosanthes cucumerina* was also evaluated using the cyclic voltammetry technique. The antioxidant capacity of the methanolic extracts of *Momordica charantia and Trichosanthes cucumerina* are shown in Table 3. The cyclic voltammograms for the two extracts are shown in Figure 3. As shown the figure, the voltammograms of the extracts all exhibited an irreversible behaviour with a well-defined anodic peak potential at ~0.89 V for *Trichosanthes cucumerina* and ~0.98 V for *Momordica charantia* vs. Ag/AgCl. Similar irreversible behaviour has been reported for the extract of *Thymus vulgaris* [52]. These irreversible anodic peaks at comparatively high potentials indicate that both fruit extracts contain effective antioxidant species that can donate electrons. The lower oxidation potential (0.89 V *vs*. Ag/AgCl) of *Trichosanthes cucumerina* than *Momordica charantia* (0.98 V *vs*. Ag/AgCl) suggests that the former has more easily oxidised antioxidant compounds. This can be buttressed with the presence of most highly oxidised antioxidants in aqueous system ascorbic acid (vitamin C) and polyphenols (phenolic compounds) contents. It suggests a better electron-donating capacity and, consequently, higher antioxidant efficiency from an electrochemical perspective. This is consistent with previous findings that antioxidant values increase with decreasing oxidation potential [26, 52]. The methanolic extract of *Trichosanthes cucumerina* had a quantitative antioxidant capacity of 1.38±0.16 µg GA Eq./mg and 4.60±0.26 µg AA Eq./mg, while *Momordica charantia* had 1.05±0.03 µg GA Eq./mg and 1.17±0.06 µg AA Eq./mg.

Table 4 shows the *Trichosanthes cucumerina (TC)* and *Momordica Charantia (MC)* Cyclic voltammetric results

Table 4: Antioxidant capacity of *Trichosanthe cucumerina Momordica Charantia* determined using the cyclic voltammetry method

Sample	µg GAE/mg	µg AAE/mg	E (V)
Trichosanthe cucumerina	1.38 ± 0.16	4.60 ± 0.26	0.89
Momordica charantia	1.05 ± 0.03	1.17 ± 0.06	0.98

These results show that, in terms of gallic acid and ascorbic acid equivalents, *T. cucumerina* antioxidant activity is higher than *Momordica charantia*. The results further suggest that *Trichosanthes cucumerina* extract contains more electroactive antioxidant compounds, particularly those that act like ascorbic acid, which is a strong natural antioxidant. The cyclic voltammograms in Figure 3 further show a higher peak current response for *Trichosanthes cucumerina* than *Momordica charantia* under identical experimental conditions.

The sharp anodic response observed in the *Trichosanthes cucumerina* extract indicates a higher concentration of redox-active compounds present that can undergo electrochemical oxidation. This trend is consistent with the findings for Herzegovinian wild flowers and *Solanum nigrum (Black nightshade)*, respectively, where extracts with lower anodic potentials and higher peak currents were found to exhibit greater antioxidant potential [24, 38].

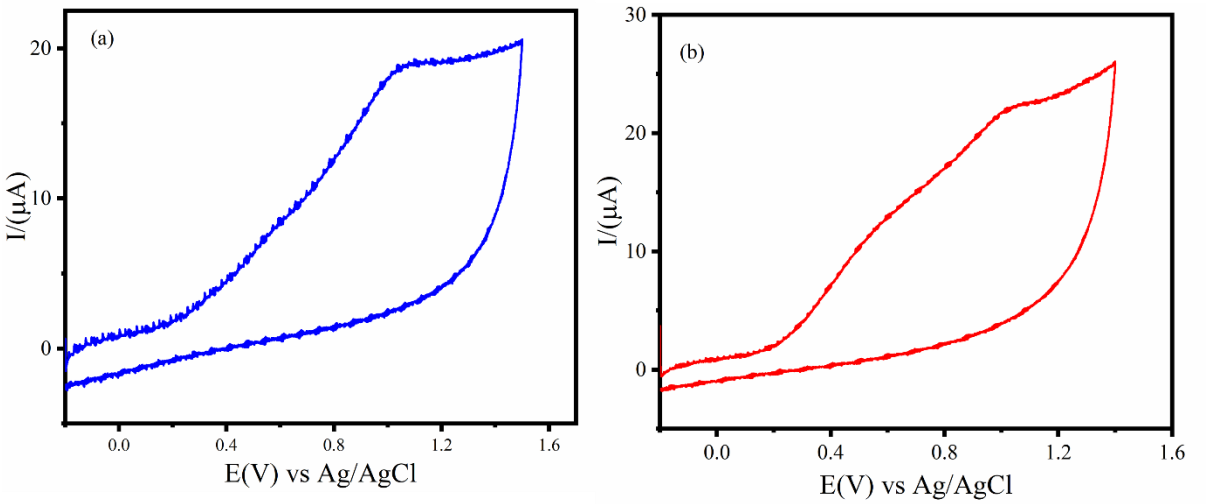

Figure 3. Cyclic voltammograms of methanolic extracts of various (a) *Momordica charantia*, (b) *Trichosanthes cucumerina*: recorded in 0.1 M phosphate buffer solution containing 0.1 M NaCl, pH 7.4, at a scan rate of 50 mV/s.

The cyclic voltammetry method shows that *T. cucumerina* has superior antioxidant capacity compared to *M. charantia*, demonstrated by its higher antioxidant values in both GAE and AAE and its lower oxidation potential. This approach offers direct insight into the redox behaviour and effectiveness of antioxidant activity in plant extracts. It serves as a reliable complement to the spectrophotometric method for assessing antioxidant capacity.

CONCLUSION

The present study evaluated the antioxidant capacity, total phenolic, and flavonoid contents of fruit extracts from *Trichosanthes cucumerina* and *Momordica charantia* using electrochemical and spectrophotometric methods. Results show that the solvent and the extraction methods affected the yield and bioactive compounds in the extracts. Microwave-assisted extraction improves the assessment of antioxidant potential in plant matrices. The fruit extracts contained significant amounts of phenolics and flavonoids. The antioxidant tests, DPPH, FRAP, and CV, indicated that *T. cucumerina* has higher antioxidant activity compared to *M. charantia*. Consequently, *T. cucumerina* could be used as a natural antioxidant in food, medicinal, and nutraceutical applications.

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