



Exploring the Possibility of Using *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf) Backeb and *Opuntia ficus-indica* (L.) MILL. as a Source of Bio-electrolyte

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Authors' contributions

This work was carried out in collaboration among all authors. Author MV performed material preparation, sample collection, and data analysis and wrote the first draft of the manuscript. Authors ON and AO reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Abstract

The global search for sustainable and affordable green energy sources has intensified due to escalating environmental concerns, health issues, energy access challenges and fossil fuel depletion. Succulent plants such as *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf) Backeb and *Opuntia ficus-indica* (L.) Mill are fast-growing, drought-resistant and invasive in nature, thriving in arid and marginal lands where food crops cannot survive. These plants store significant amounts of water, bioinorganic and bio-organic matter, making them promising candidates for bio-electrolyte feedstock in renewable bio-fuel production.

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Currently, these plants do not have any significant use, yet they are invasive and are rapidly spreading, killing pasture for livestock in rural areas. This study investigated the potential of *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf) and *Opuntia ficus-indica* (L.) Mill saps as plant-based bio-electrolytes. Sap pH and electrical conductivity were measured at different temperatures and dilution levels, while voltage and current were measured using Zn-Cu, Zn-C, Zn-Fe, Fe-Cu, Fe-C and Cu-C electrode pairs. Pearson correlation and general linear model analyses were used to examine relationships among the measured variables. The results showed that pH and electrical conductivity decreased with an increase in temperature. Voltage showed relatively small changes during the short-term monitoring period but varied among plant species and electrode-pair combinations. Applied voltage had a weak positive correlation with current ($r = 0.153$, $p < 0.001$), while the Fe-Cu electrode pair recorded the highest estimated mean current of 3.66 mA. Overall, the results provided preliminary evidence that the three plant saps can function as bio-electrolytes in electrochemical cells.

Keywords: Bio-electrolyte; electrodes; pH; conductivity; bio-battery; digital meter; plant sap.

1. Introduction

The spread of invasive succulent plants has become an increasing environmental challenge in many regions, particularly in areas where these species can survive under harsh climatic conditions. In Kenya, where approximately 80% of the landmass consists of arid and semi-arid areas and pastoralism remains a major economic activity, invasive cactus species have significantly affected grazing systems and natural ecosystems. Among these species, *Opuntia ficus-indica* (L.) Mill has received considerable attention because of its rapid expansion and negative impact on biodiversity and livelihoods. The plant is largely considered undesirable due to its sharp spines and fine hair-like glochids, which cause injuries to humans. In Narok County, the species has spread extensively over the past half-decade, resulting in the displacement of communities and a reduction in grazing area (Githae, 2018). Similarly, its spread in Laikipia County over the past decade has affected grazing lands and contributed to negative impacts on ecosystems and livestock (Shackleton et al., 2017). Within the Serengeti-Mara ecosystem, invasion by *Opuntia ficus-indica* (L.) Mill has contributed to the displacement of native plant species (Witt et al., 2017). In addition, approximately 500 km² of the area surrounding Tsavo East National Park has been affected by the invasion of this species (CABI, 2018), while in Naibunga Conservancy, the plant has spread over approximately 17000 acres and continues to expand annually by about 2km into its surrounding settlement areas (Daily Nation, 2017). The continued expansion of invasive succulent species has resulted in ecological and socioeconomic challenges, including loss of grazing areas, replacement of valuable pasture species and restricted livestock movement (Maundu et al., 2009). Their ability to tolerate environmental stress enables them to compete effectively with native vegetation, resulting in changes in plant community composition (Ivens, 1989). Once established, invasive plants can influence biodiversity, nutrient cycling, water availability, decomposition processes and other ecological functions (Pimentel, 2002; Oba et al., 2000). Globally, invasive plant species contribute to substantial economic losses estimated at billions of dollars due to their effects on agriculture, ecosystems and natural resources (Richardson et al., 2000; Wilcove et al., 1998; Yurkonis et al., 2005). Among these species, *Opuntia stricta* has been identified as one of the most problematic species affecting ecosystems and agricultural production systems in different regions (Bright, 1998). The cactus species *Opuntia ficus-indica* (L.) Mill, *Opuntia stricta* and *Opuntia monacantha* were introduced in Kenya during the 1950s by colonial stakeholders (Strum et al., 2015). These species belong to the Cactaceae family and originated from Central America before becoming established in different parts of the world, including South Africa, India, Australia and Mediterranean regions (Aruwa et al., 2018). Their successful establishment is associated with unique physiological adaptations, particularly Crassulacean Acid Metabolism (CAM), which improves water-use efficiency. CAM plants are able to produce greater biomass with reduced water loss compared with plants using C3 and C4 photosynthetic pathways (Skogberg et al., 2025).

Succulent plants are well known for their ability to survive under harsh environmental conditions because of their efficient water-storage capacity and specialized physiological adaptations (Brickell, 1999). Among them, *Agave sisalana* Perrine, which originated in Mexico and Central America (Pandey, 2003), is widely recognized for its strong leaf fibers and its contribution to soil conservation, rehabilitation of degraded land and erosion control (Huxley et al., 1992; Nobel, 1994; Abdel-Hady et al., 1995). Similarly, *Opuntia* species provide food, livestock forage and protection against soil erosion in arid and semi-arid regions, making them valuable resources. Beyond their agricultural and ecological importance, succulent plants have recently attracted attention

as potential sources of bio-electrolytes. The high moisture content of these tissues, together with the presence of naturally occurring ions and organic compounds, suggests that their sap may support electrochemical reactions. At the same time, increasing environmental concern associated with fossil fuel use, including greenhouse gas emissions, air pollution, rising energy costs and climate change, has accelerated the search for cleaner and more sustainable energy technologies (International Monetary Fund, 2000; Winters et al., 2002; Azar et al., 2003; Khan et al., 2011). Consequently, renewable energy systems with low carbon emissions have become an important research priority (Panwar et al., 2011; Swift-Hook, 2013; Arent et al., 2011). Among these emerging technologies, bio-electrochemical systems have attracted considerable interest because they convert chemical energy stored in biological materials into electrical energy through oxidation-reduction reactions occurring at the electrode-electrolyte interface (Larkum, 2010). Measurements of plant electrical properties have shown sensitivity to water status, light and temperature (Sinha & Tabib-Azar, 2015), while electrochemical-cell development studies underscore the importance of cell construction and material selection for controlled testing (Jimenez-Hernandez et al., 2025). Previous studies have shown that electricity can be generated from plant materials and extracts obtained from vegetable peels, leaves and flowers, which can function as electrolytes in simple galvanic cells (Nath & Nath, 2015). Recent studies on renewable biopolymer electrolytes have further demonstrated the electrochemical relevance of cellulose and plant gums as ion-conducting matrices for battery applications (Li et al., 2024; Vengadesh Krishna et al., 2025). These findings demonstrate the feasibility of plant-derived bio-electrolytes, but they also reveal several gaps in current knowledge. Most previous studies have focused primarily on demonstrating electricity generation from plant materials, while comparatively little attention has been given to understanding how the physicochemical properties of plant sap influence electrochemical performance. In particular, the effects of temperature on pH and electrical conductivity, the relationship between these properties and electrical output, and the influence of different electrode combinations have not been investigated systematically for succulent plant saps. In addition, comparative studies involving multiple succulent species under identical experimental conditions remain limited.

The present study addresses these gaps by investigating the physicochemical and electrochemical properties of sap extracted from *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf.) Backeb, and *Opuntia ficus-indica* (L.) Mill. Specifically, the study evaluates the influence of temperature on pH and electrical conductivity, examines voltage stability over time using different electrode combinations, and investigates current-voltage characteristics to assess the electrochemical behavior of the sap-based electrolytes. By combining physicochemical characterization with electrochemical evaluation, this work provides a broader understanding of the suitability of invasive succulent plants as bio-electrolytes and contributes information that may support the development and optimization of sustainable plant-based bio-battery systems. Figure 1 illustrates the succulent species investigated in this study in their natural habitats.



Fig. 1. (a) *Agave sisalana* Perrine, (b) *Opuntia ficus-indica* (L.) Mill, (c) *Austrocylindropuntia subulata* (Muehlenpf.) Backeb plants in their natural habitats in Narok county, Kenya

2. Methodology

Plant leaves of *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf.) Backeb and *Opuntia ficus-indica* (L.) Mill were collected from wild populations in the Mt. Suswa area (1.1782 ° S, 36.33 ° E), Narok County, Kenya. The plant materials were collected in accordance with local, institutional, national and international guidelines. Plant identification and authentication were performed by Justus Kikuvi (Head, Botany Department, National Museum of Kenya), and samples were deposited there.

Voucher specimens of *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf.) Backeb and *Opuntia ficus-indica* (L.) Mill were deposited at the East African Herbarium (EA), Nairobi, Kenya, under the voucher numbers below.

2.1 Voucher Information

- 1) *Agave sisalana* Perrine; Mutindi V. No.001, deposited at East African Herbarium.
- 2) *Austrocylindropuntia subulata* (Muehlenpf.) Backeb.; Mutindi V. No 002, deposited at East African Herbarium.
- 3) *Opuntia ficus-indica* (L.) Mill.; Mutindi V. No 003, deposited at East African Herbarium.

Fresh, mature leaves of selected succulent species were harvested, rinsed with distilled deionized water, and macerated to obtain sap, which was filtered through a muslin bag and Whatman No. 1 paper. Sap was stored in airtight containers at a temperature of 21±2°C and analyzed within 2 hours after extraction to minimize possible changes associated with storage. The sap was used without dilution in all physicochemical and electrochemical measurements. The pH of distilled deionized water was measured to be 7.00.

2.2 pH and Conductivity

pH and electrical conductivity (EC) were measured using a Universal Multiline Hanna pH 210 Microprocessor (Hanna G-114, Germany), a calibrated digital meter with temperature compensation, and an INE-EC110B Portable Conductivity EC Meter for measurement of electrical conductivity. Measurements were performed at a laboratory temperature of 21±2°C. Instrument calibration was performed before analysis using standard calibration solutions. To evaluate the influence of temperature on sap properties, sap aliquots (50 mL) were heated stepwise from 20°C or 21°C to 41°C using a thermostatic water bath. Measurements were taken at 1 °C intervals. Subsequently, 10 -40 ml of distilled deionized water were added to each neat sap of the three plant species, and temperature was measured at 1 °C intervals at each dilution. The pH of distilled deionized water was measured before it was added to the sap. All measurements were conducted in triplicate, and average values were used for subsequent analysis.

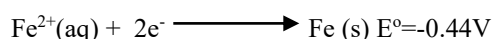
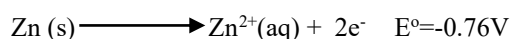
2.3 Voltage–Time

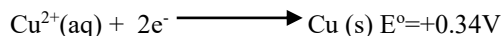
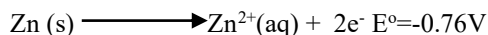
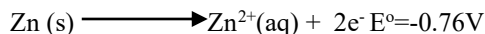
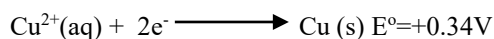
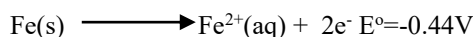
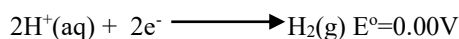
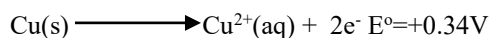
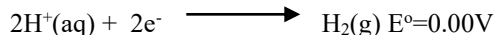
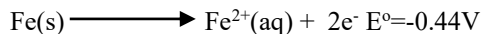
A two-electrode cell (copper, carbon, iron or zinc electrodes, 3 cm spacing) containing 50 mL sap was connected to a Sanwa CD800a digital multimeter for voltage measurements. Open-circuit voltage was recorded immediately for 120 min at room temperature for each of the three plant saps. All measurements were conducted in triplicate, and average values were used for subsequent analysis.

The electrochemical cell consisted of 50ml of plant sap contained in a plastic beaker; a pair of electrodes was immersed vertically with an electrode spacing of 3cm.

2.4 Electrode Reaction

Zn-Fe cell



Zn-Cu cell**Zn-C cell****Fe-Cu cell****Cu-C cell****Fe-C cell**

Dimensions of the electrode:

Electrode type	Dimensions	Exposed surface area
Copper	8.0cm × 1.5cm × 1.0mm	18cm ²
Zinc	5.5cm × 3.5cm × 1.0mm	24.5cm cm ²
Iron	8.5cm × 1.5cm × 1.0mm	18 cm ²
Carbon	7.5cm × 1.5cm × 3mm	16.5 cm ²

Before each experiment, metallic electrodes were polished using fine abrasive paper, rinsed with distilled deionized water, cleaned with ethanol and allowed to air-dry before use. Carbon electrodes were rinsed thoroughly with distilled deionized water and allowed to air-dry before being immersed in the plant sap.

2.5 Current–Applied voltage (I–V)

The cell for each of the three plants, containing 50ml of sap, was connected to a UTP3316TFL-11 REGULATED DC power supply, and voltage was varied from 0– 31.5V, with a maximum voltage reading of 31.5V and 10–30 s stabilization at each step before current was recorded in triplicate while room temperature was maintained constant. All measurements were conducted in triplicate, and average values were used for subsequent analysis.

3. Results and Discussion

The data obtained in this study showed that the three plant samples can be used as bio-electrolytes. The results focused on the effect of temperature variation on plant-sap pH and electrical conductivity, voltage generation from the three plant saps when different electrode pairs were used, and the effect of the current-applied voltage relationship on the three plant saps.

Together, these results enable an assessment of the suitability of *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf.) Backeb and *Opuntia ficus- indica* (L.) Mill saps for bio-battery applications.

3.1 Temperature Dependence of Sap pH

Figs. 2–4 show the variation in pH with temperature for the sap extracts of *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf.) Backeb, and *Opuntia ficus- indica* (L.) Mill.

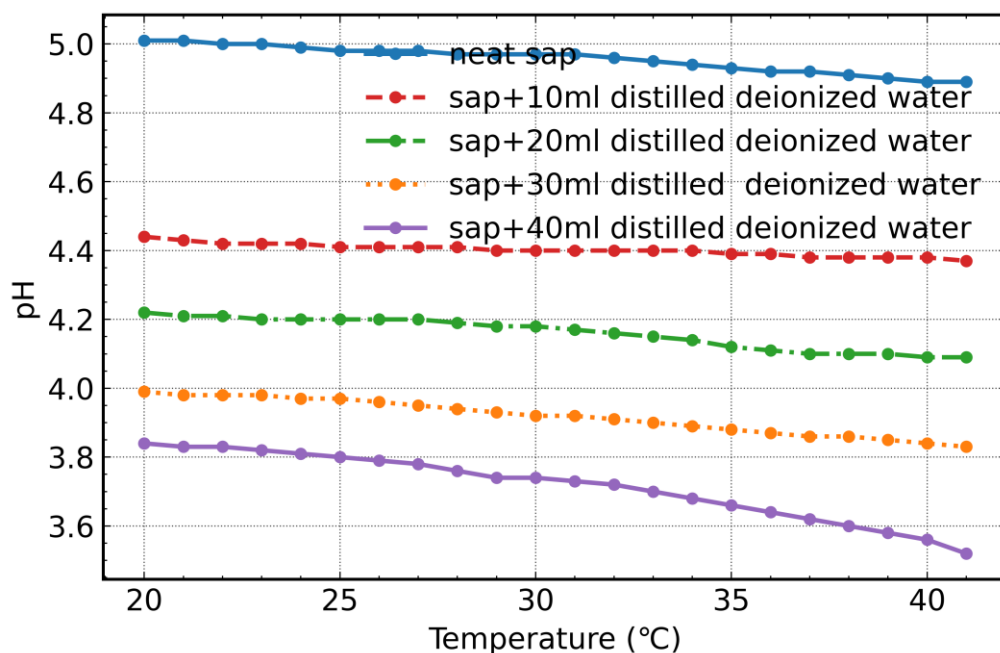


Fig. 2. pH measurement against temperature for *Agave sisalana* Perrine sap with dilution with distilled deionized water

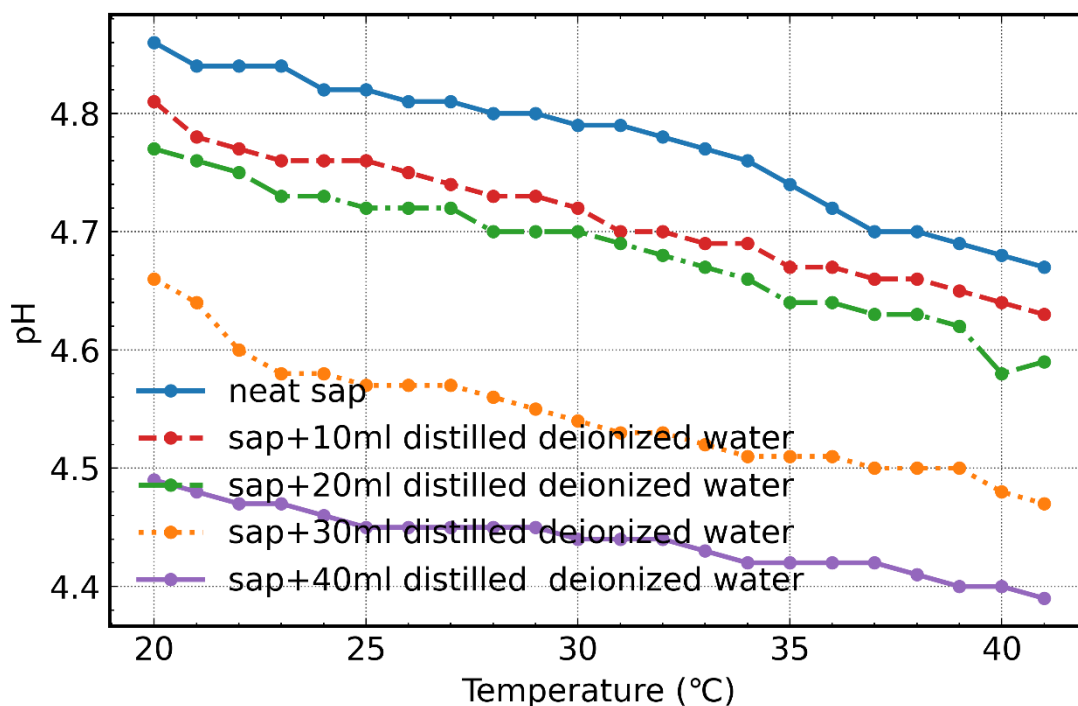


Fig. 3. pH measurement against temperature for *Austrocylindropuntia subulata* (Muehlenpf.) Backeb sap with dilution with distilled deionized water

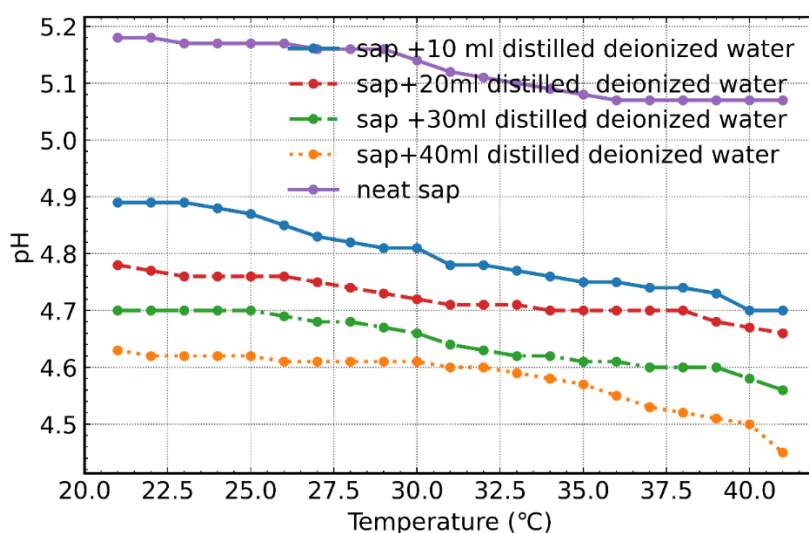


Fig. 4. pH measurement against temperature for *Opuntia ficus-indica* (L) Mill sap with dilution with distill deionized water

Figs. 2-4 show that increasing temperature led to a decrease in the pH of the different plant saps, indicating a shift towards more acidic conditions. This trend may be attributed to the enhanced ionisation of weak organic acids present in the sap at elevated temperatures, which increases hydrogen ion concentration and consequently lowers pH, as suggested by Carreón-Hidalgo et al. (2023) and Coşkuner et al. (2000). It might also be due to thermal degradation of natural buffering compounds, thereby reducing the sap's capacity to maintain pH stability under heat stress, as reported by Wisutitprot et al. (2022). Finally, the sap contains temperature-sensitive polysaccharides and phenolic compounds that become unstable at higher temperatures, further contributing to increased acidity, as reported by Antony and Farid (2022). Overall, the consistent decrease in pH with rising temperature across all sap samples highlights the susceptibility of succulent plant saps to thermally induced chemical change.

3.2 Temperature and Dilution Effects on Electrical Conductivity of Plant Saps

Figs. 5–7 illustrate the relationship between temperature and electrical conductivity for the different plant saps.

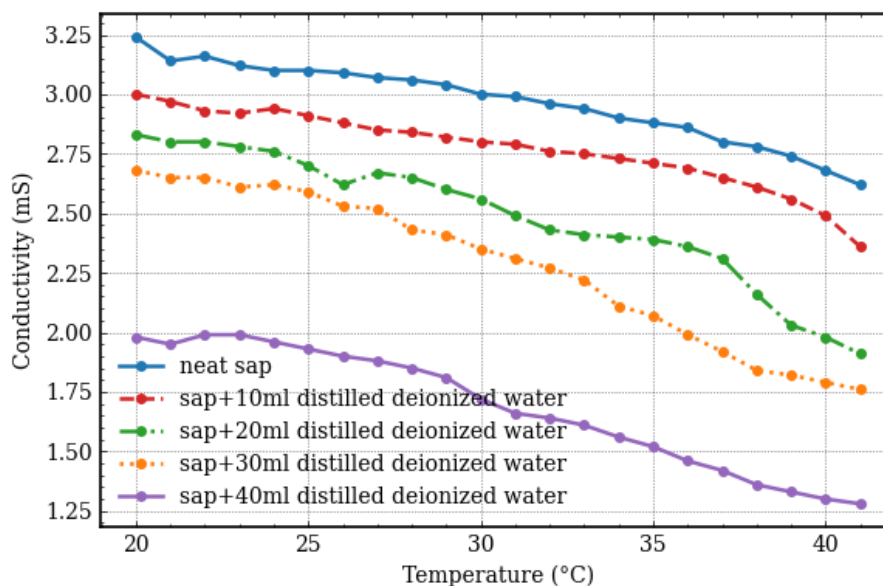


Fig. 5. Conductivity measurements against temperature for *Agave sisalana* Perrine sap with dilution with distill deionized water

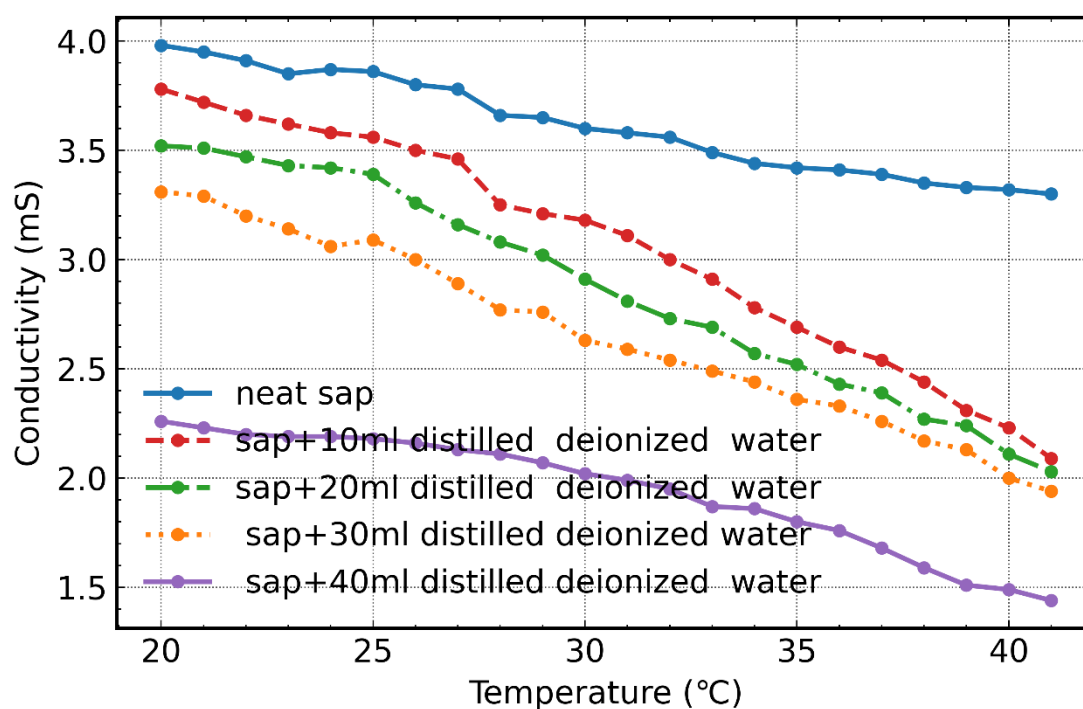


Fig. 6. Conductivity measurement against temperature for *Austrocylandropuntia subulata* (Muehlenpf.) Backeb sap with dilution with distilled deionized water

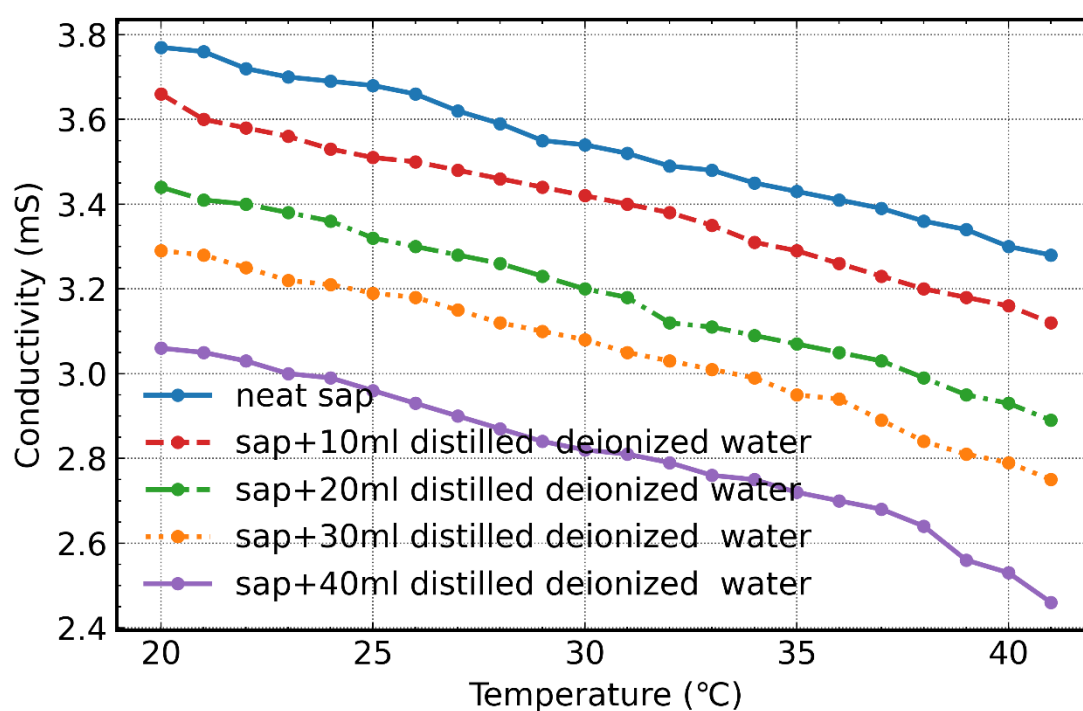


Fig. 7. Conductivity measurement against temperature for *Opuntia ficus-indica* (L.) Mill sap with dilution with distilled deionized water

Figs. 5–7 illustrate the variation in electrical conductivity with temperature for *Agave sisalana* Perrine, *Austrocylandropuntia subulata* (Muehlenpf.) Backeb, and *Opuntia ficus-indica* (L.) Mill saps at different serial dilution levels. Across all plant species, electrical conductivity decreased linearly with increasing temperature

over the studied range of approximately 20–41 °C. This temperature-dependent decline in conductivity contrasts with the behavior of conventional electrolytes and indicates that elevated temperatures adversely affect ionic conduction within plant sap electrolytes. This may be attributed to thermally induced structural changes in polysaccharides and other macromolecular constituents of the saps, which restrict ion mobility, as previously suggested by Berradi et al. (2023) and Alavarse et al. (2022). In addition, weakening of intermolecular and ion–solute interactions within the sap matrix reduces viscosity-related transport efficiency and promotes partial degradation of ionic species, collectively limiting effective charge transport, as reported by Mizuhata (2022) and Barclay et al. (2025). The neat sap recorded the highest conductivity, and dilution with distilled deionized water decreased conductivity; this was attributed to decreased ionic strength and buffering capacity. *Austrocylindropuntia subulata* (Muehlenpf.) Backeb recorded the highest conductivity, followed by *Opuntia ficus-indica* (L.) Mill and finally *Agave sisalana* Perrine; this was attributed to sap organic molecule composition (Mizuhata, 2022; Barclay et al., 2025).

3.3 Voltage Stability over Time

Figs. 8–10 present voltage–time characteristics for the different plant saps using various electrode combinations. Figs. 11–13 present the voltage–time profiles for five electrode pairs (Zn–C, Zn–Cu, C–Fe, Zn–Fe and Cu–Fe) across different plant saps. Overall, voltage variation over the experimental duration was minimal for all electrode combinations, indicating relatively stable electrochemical behavior.

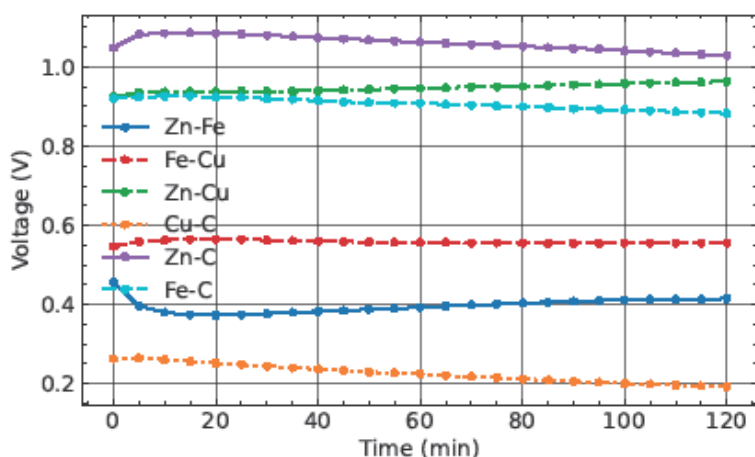


Fig. 8. Voltage measurement against time for *Agave sisalana* Perrine sap using different pair electrodes

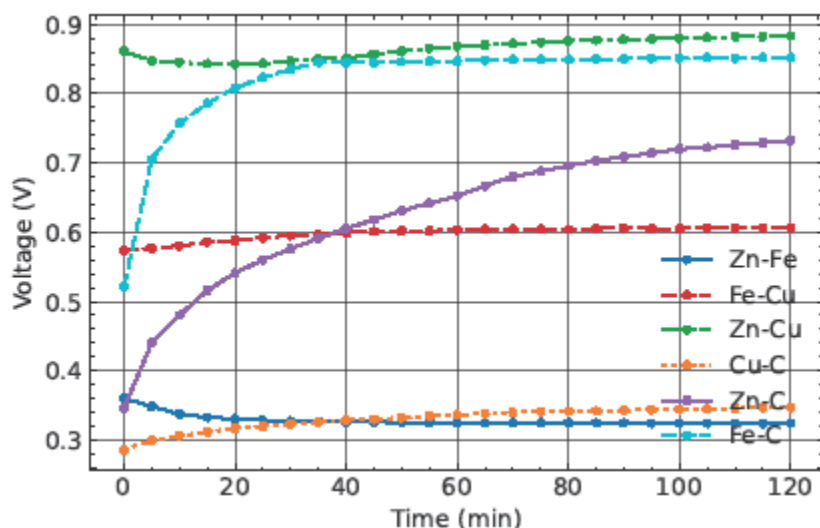


Fig. 9. Voltage measurement against time for *Austrocylindropuntia subulata* (Muehlenpf.) Backeb sap using different pair electrodes

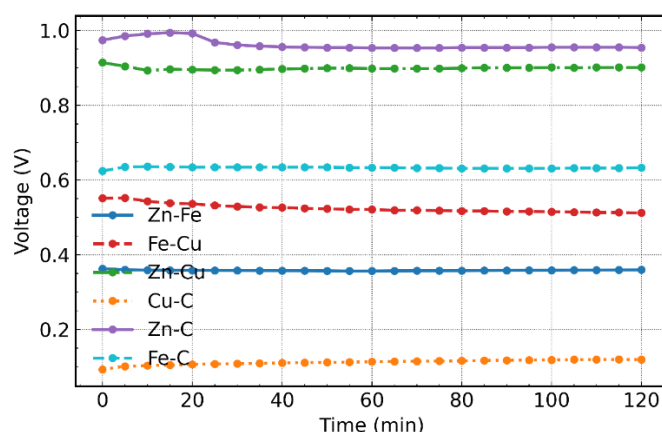


Fig. 10. Voltage measurement against time for *Opuntia ficus-indica* (L.) Mill sap using different pair electrodes

Figs. 8–10 illustrate the voltage-time behavior of different electrode pairs immersed in plant sap electrolytes. Generally, the voltage output for all electrode combinations exhibited only minor fluctuations over a 120-minute monitoring period, indicating good short-term electrochemical stability of the sap-based bio-electrolyte pairs. The results also showed that the Zn–Cu and Zn–C electrode pairs consistently produced the highest voltage, followed by C–Fe, then Cu–Fe, and finally Zn–Fe and Cu–C electrode pairs, which recorded the lowest voltage output. Voltage variation for each electrode pair remained minimal throughout the experimental period, reinforcing the stability of the bio-electrolyte system. These results are closely similar to those of Phiri et al. (2021). The observed influence of plant species further suggests that electrolyte composition plays a critical role in determining electrical output. The voltage measurements consisted of 450 observations collected over a 120-minute monitoring period. The mean voltage was 0.621 ± 0.280 V, while the mean monitoring time was 60.0 ± 36.1 minutes. Pearson correlation analysis showed a weak positive relationship between mean voltage and time, where $r=0.022$, 95% CI: $-0.070-0.115$, $p=0.636$. This suggested that there was no significant linear relationship between time and voltage when the two variables were considered. The general linear model showed significant effects of time, plant species, electrode pair and the interaction between plant species and electrode pair on voltage. Time had a significant effect on voltage, where $F=15.866$, $p<0.001$, partial $\eta^2=0.036$. Plant species had a significant effect, $F=400.440$, $p<0.001$, partial $\eta^2=0.650$, while electrode pair had a large effect, where $F=5453.839$, $p<0.001$, partial $\eta^2=0.984$. The interaction between plant species and electrode pair was also significant, where $F=310.196$, $p<0.001$, partial $\eta^2=0.878$. These results indicated that voltage output varied considerably according to the plant species and electrode combination used.

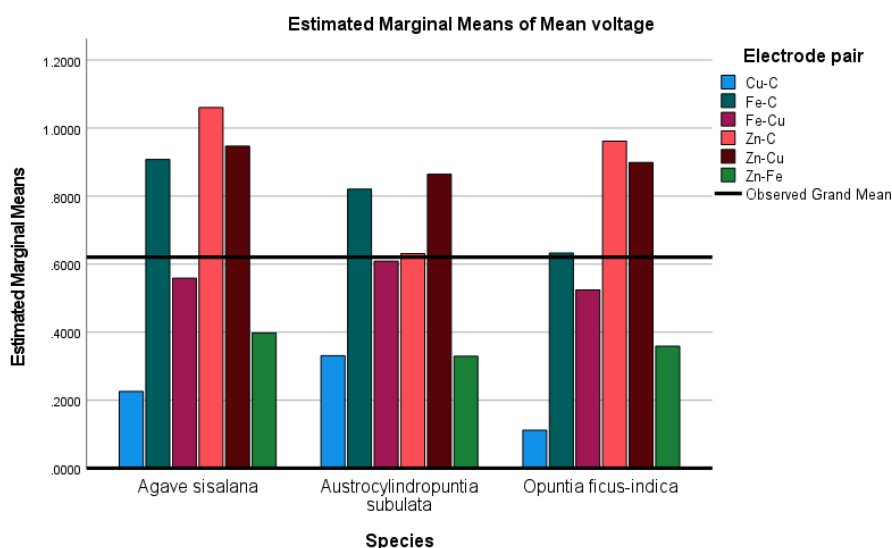


Fig. 11. Estimated marginal means of voltage across three plant species showing interaction between plant species and electrode pair

From the observed mean values, *Agave sisalana* generally produced the highest voltage across the electrode combinations. The Zn-C electrode pair recorded the highest mean voltage, exceeding 1.0V, followed by Zn-Cu, which recorded approximately 0.93V. The Cu-C pair produced the lowest mean voltage at approximately 0.22V. The relatively small changes observed during the monitoring period suggested that each electrode-pair combination maintained its voltage output over the monitoring period, although long-term studies would be required to determine the stability of the system.

3.4 Current–Applied Voltage Characteristics

Figs. 12–14 show the current–voltage relationships for the three plant saps.

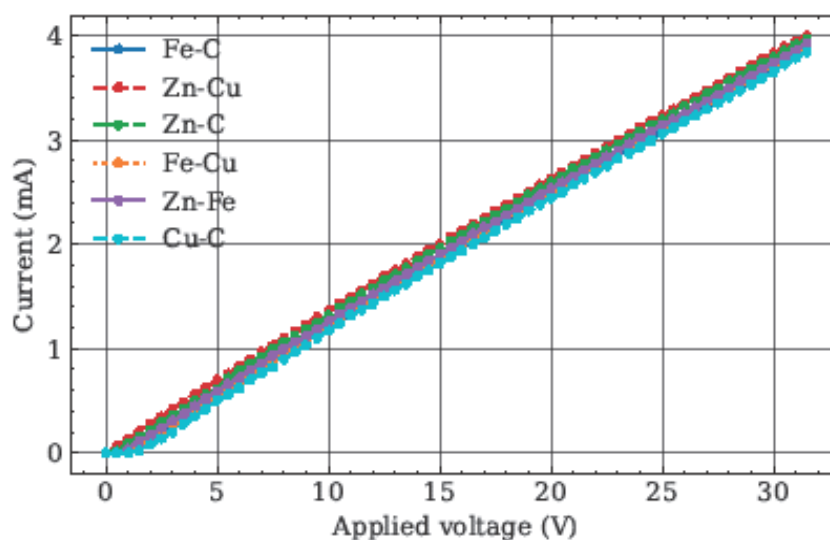


Fig. 12. Current measurement against applied voltage for *Agave sisalana* Perrine sap using different electrode

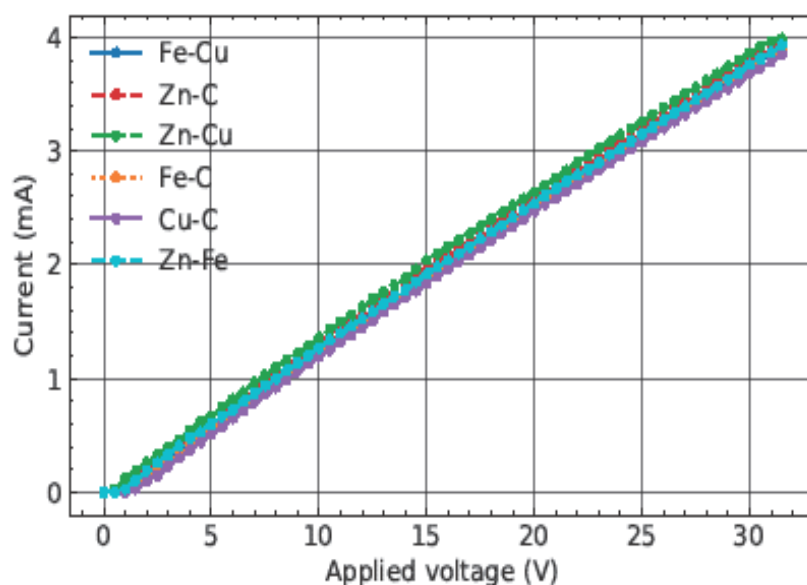


Fig. 13. Current measurement against applied voltage for *Austrocylindropuntia subulata* (Muehlenpf.) Backeb sap using different electrode

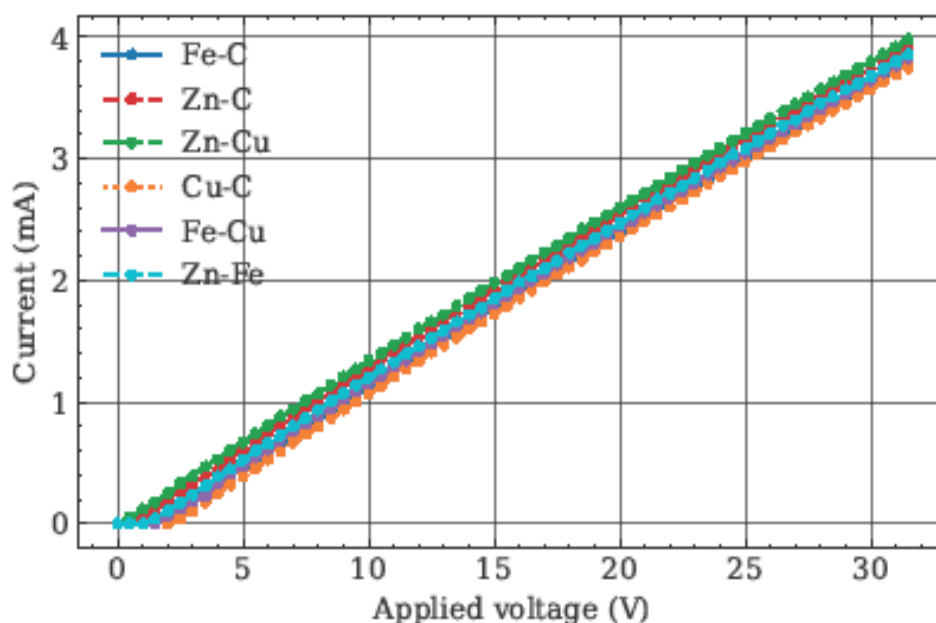


Fig. 14.Current measurement against applied voltage for *Opuntia ficus- indica* (L.) Mill sap using different electrode

Figs. 12-14 illustrate that the externally applied DC voltage was not intended to measure electrical power generated by the bio-electrolyte. Rather, it was used to characterize the electrical response of the plant sap under controlled polarization conditions. It can be observed that current increased with variation in applied voltage. There was a small variation in current among different electrode pairs. Among the different electrode pairs used, the Fe–Cu electrode pair produced the highest current output, followed by the Zn–Cu electrode pair, the Zn–C electrode pair, the Zn–Fe electrode pair, the Fe–C electrode pair and, finally, the Cu–C electrode pair, which consistently recorded the lowest current. The ranking highlights the influence of electrode material on charge-transfer efficiency in plant-based electrochemical systems. For *Austrocylindropuntia subulata* (Muehlenpf.) Backeb sap, the current increased from 0 mA at 0 V to a maximum of 3.96 mA at 31.5 V. Similarly, *Agave sisalana* Perrine sap exhibited a current increase from 0 mA at 0 V to 3.95 mA at 31.5 V, while *Opuntia ficus-indica* (L.) Mill sap recorded a rise from 0 mA at 0 V to 3.92 mA at 31.5 V. Overall, Figs. 12–14 reveal a linear current–voltage relationship for all plant saps, consistent with ohmic behavior, and confirm that the bio-electrolytes support efficient and stable sap charge-transport mechanisms, as reported previously by Kumar et al. (2012). The maximum currents observed ranged between 3.9 and 4.0 mA at 31.5 V, indicating that the electrochemical performance of the three plant saps is comparable, as found by Paul et al. (2023). The superior current output recorded with Fe–Cu, Zn–C and Zn–Cu electrode pairs reinforces their suitability for bio-battery and bio-electrochemical energy conversion applications, in agreement with recent findings reported by Pan et al. (2025). The relationship between applied voltage and current was evaluated using Pearson correlation analysis. A total of 1152 observations were used to examine the relationship between applied voltage and current. The mean applied voltage was 15.75 ± 9.24 V, while the mean current was 2.24 ± 9.94 mA. Pearson correlation analysis showed a weak but positive relationship between applied voltage and current, $r=0.153$, 95% CI, 0.097-0.209, $p<0.001$; although the relationship was statistically significant, its strength was weak. The value of $r^2=(0.153)^2=0.023$ indicated that approximately 2.3% of the variation in current was associated with its linear relationship with applied voltage. This suggested that other factors, such as electrode pair, plant species, electrode interactions and electrolyte composition, likely contributed more strongly. The general linear model showed that applied voltage had a significant effect on the response measured in the model, $F=27.723$, $p<0.001$, $\eta^2=0.240$. However, plant species did not show a significant effect, $F=1.099$, $p=0.333$, and neither did electrode pair, $F=0.972$, $p=0.434$. The interaction between plant species and electrode pair was not significant, $F=1.008$, $p=0.434$. Therefore, although some differences could be seen in the mean current values among plant species and electrode pairs, these differences were not statistically significant in the analysis.

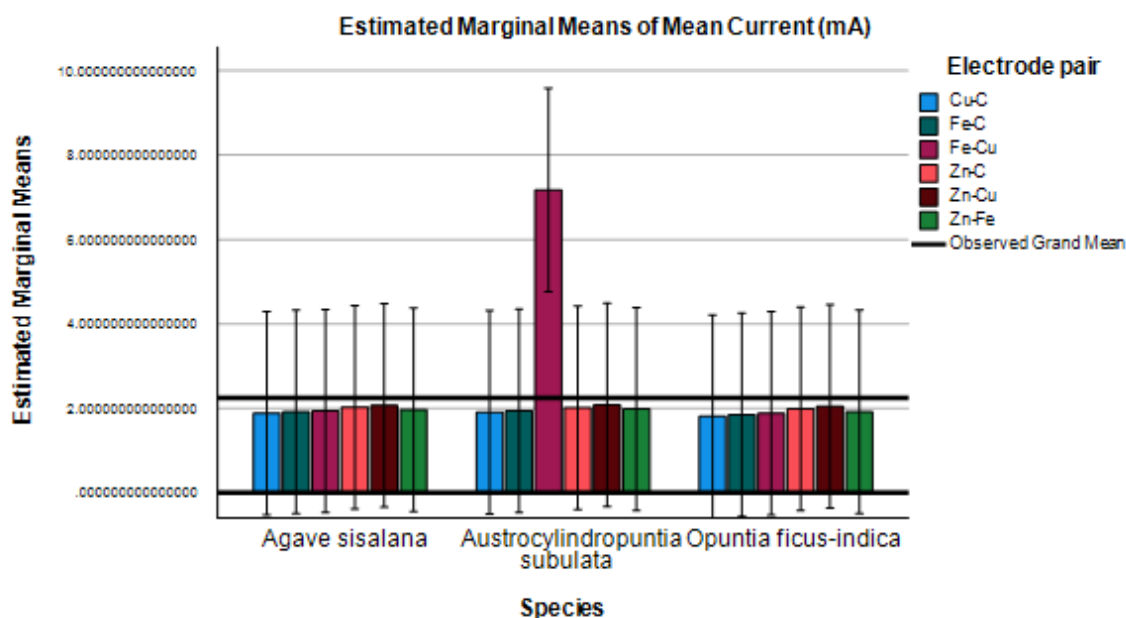


Fig. 15. Estimated marginal means of current of three plant species showing interaction between plant species and electrode pair

From Fig. 15, *Austrocyllindropuntia subulata* with the Fe-Cu electrode pair showed a spike, reaching an estimated marginal mean current of 6.8mA. For almost all other combinations across the three plant species, the mean current remained uniform. *Agave sisalana* showed consistent current generation (1.8-2.0mA) across the six electrode pairs, with low variation between the electrode pairs. *Opuntia ficus-indica* exhibited the lowest overall current output among the three plant species, ranging slightly from 1.5-1.8mA across the electrode pairs. The mean current recorded across all experimental observations was 2.24 ± 9.94 mA. The relatively large standard deviation compared with the mean indicated variation in current measurements across the different applied voltages, plant species and electrode-pair combinations. The estimated mean current also varied among the electrode pairs, with the Fe-Cu electrode pair showing the highest mean current of 3.36mA, followed by Zn-Cu (2.06mA), Zn-C (2.01mA), Zn-Fe (1.95mA), Fe-C (1.90mA) and, finally, Cu-C, which recorded 1.86mA.

4. Limitation of the Study

The current study has some limitations that should be considered when interpreting the results. The electrochemical analysis was mainly based on measurements of voltage, current, electrical conductivity and pH. These measurements provided useful information about the electrical behavior of the plant saps, although they did not capture the electrochemical processes taking place at the electrode-electrolyte interface. More detailed electrochemical measurements would be useful in future studies to understand the charge-transfer process. Other limitations included the short-term duration of the electrical measurements; the study did not establish how well performance would be maintained over longer periods. Therefore, long-term experiments would be necessary to determine changes in electrical output over prolonged operation and the durability of the electrode-electrolyte systems. The study did not provide an assessment of power density and energy density; therefore, further investigations should include power- and energy-density measurements under controlled load conditions. Plant sap is a biological material, and its composition can vary naturally. Plant age, environmental conditions, physiological state, harvesting time and sample handling could influence the concentration of ions and contribute to variation in pH, conductivity and voltage; therefore, better standardization of sampling and preparation would be helpful. The chemical composition of the electrolyte was not fully determined in this study; therefore, chemical characterization of the electrolyte would be important to identify the components that contribute to ionic conduction and electrical performance.

The current-voltage measurements were performed using applied voltage. This approach was useful for examining the electrical response of the sap and electrode combinations, but it does not represent the same

operating conditions as a self-powered bio-battery and may have been influenced by electrode polarization, contact resistance and the configuration of the experimental system.

5. Conclusion

This study evaluated the physicochemical and electrochemical behavior of saps from *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf.) Backeb, and *Opuntia ficus-indica* (L.) Mill as potential bio-electrolytes. Within the studied temperature range, pH and electrical conductivity decreased as temperature increased, while dilution reduced electrical conductivity. Voltage remained comparatively stable during the 120-min monitoring period, although its magnitude varied with plant species and electrode pair. Statistical modelling indicated significant effects of plant species, electrode pair and their interaction on voltage. Applied voltage was positively but weakly correlated with current, and differences in current among plant species and electrode pairs were not statistically significant in the reported model. These findings indicate that the three plant saps can support ionic conduction and electrochemical responses under the investigated laboratory conditions. Their application as practical bio-electrolytes remains preliminary. Further studies should assess long-term stability, electrolyte composition, electrode configuration, reproducibility, current density, power density and energy density under controlled load conditions before their suitability for bio-battery applications is established.

Ethical Approval

The collection and use of plants material complied with the relevant locally, institutional, national and international guidelines.

Plant leaves of *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf.) Backeb and *Opuntia ficus-indica* (L.) Mill were collected from wild populations in the Mt. Suswa area (1.1782 ° S, 36.33 ° E) Narok county, Kenya. The plants material were collected in accordance with locally, institutional, national and international guidelines. Plants identification and authentication were performed by Justus Kikuvi (Head, Botany Department, National Museum of Kenya and samples were deposited there.

Voucher specimens of *Agave sisalana* Perrine, *Austrocylindropuntia subulata* (Muehlenpf.) Backeb and *Opuntia ficus-indica* (L.) Mill were deposited at the Herbarium East African Herbarium (EA) Nairobi, Kenya under voucher numbers below.

Voucher Information

- 1) *Agave sisalana* Perrine; Mutindi V. No.001 deposited at East African Herbarium.
- 2) *Austrocylindropuntia subulata* (Muehlenpf.) Backeb.; Mutindi V. 002 deposited at East African Herbarium
- 3) *Opuntia ficus-indica* (L.) Mill.; Mutindi V. 003 deposited at East African Herbarium

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Data availability

The remaining data used to plot the graphs are provided in the supplementary information, Tables 1-12.

Supplementary Materials

Supplementary materials available in this link:
<https://drive.google.com/file/d/1UICIsVwAat2P8GXoPq6iEP6hreRTLcFH/view?usp=sharing>

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Declaration of AI Use

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Competing Interests

Authors have declared that no competing interests exist.

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