Chapter

Electrochemical Response of Cells Using Bioactive Plant Isolates

Elvis K. Tiburu, Richard Asiamah, Bernard O. Asimeng, Samuel Kojo Kwofie, Emmanuel Nyankson and William N. Gblerkpor

Abstract

Traditional herbal medical practices continue to be part of the healthcare needs of the world especially residents of sub-Sahara Africa (sSA). However, the mechanism of action of the plant metabolites to elicit their potency continue to be a mystery due to the lack of standardized methods. The mechanism of plant bioactive compounds to cause cell death is gradually being linked to membrane polarization and depolarization behaviour. The current work seeks to probe the electrochemical response of model cells using bioactive compounds captured in bio-zeolites or membrane mimetics. The voltage and current fluctuations emanating from such studies will establish a correlation between cell death and membrane depolarization. It will be a useful biological interface sensing material with the potential to identify plant metabolites that can selectively detect and destroy diseased cells. Several model membranes have already been developed for biomedical applications and this new paradigm will elevate the usefulness of these model systems. The concept was investigated using extracts from *Dioclea reflexa (DR)* hook which belongs to the leguminous family. There are certain class of compounds in *Dioclea reflexa (DR)* that have clinical usefulness in both temperate and tropical regions, however the identity of the bioactive compounds responsible for inducing cell death continue to be a major challenge.

Keywords: model membrane, electrochemical, bioactive, polarization, depolarization, bio-zeolites

1. Introduction

Living organisms use small molecules including membrane targeted drugs to enhance membrane fluidity and permeability to elicit their potency in signal transduction as well as in the treatment of various diseases including cancer, fungal and microbial pathogens. Electrochemical signaling from reactive oxygen species (ROS) is a major mechanism used to regulate different cancer-related processes including cell proliferation, migration, invasion, metastasis and vascularization. The key players in the redox microenvironment of the cancer and neighboring cells are superoxide (O^2), hydrogen peroxide (H_2O_2), nitric oxide (NO) and ions which are produced or regulated by membrane bound nicotinamide adenine dinucleotide phosphate (NADPH), oxidases known as NOX and by the dual oxidases (DUOX),

or nitric oxide synthases. For example studies using scanning electrochemical microscopy (SECM) and fluorescence microscopy confirmed the release of ROS from prostate cancer (PC3) cells [1, 2].

Among the ion channels that are membrane bound include voltage-gated Na_+ channels that selectively allow the passage of Na^+ ions into cells resulting in membrane depolarization leading to generation of action potential in excitable cells including neurons, heart and skeletal tissues. It is known that strongly metastatic prostate cancer cell lines such as PC3 cells demonstrate significantly higher expression of voltage-gated Na^+ channels (Nav1.9 alpha subunit). Studies have shown that inhibiting specific voltage-gated Na^+ channels activity have helped to reduce cell proliferation and therefore such channels including K^+, Ca^{2+}, and Cl^- may emerge as novel biomarkers and therapeutic targets for certain cancer treatments. The concept is to develop small molecular probes or nanoparticles that are either delivery vehicles or the nanoparticle itself having the potential to specifically block a particular ion channel to prevent the movement of ions across the membrane which is key critical step for tumor cell survival.

Saccharomyces cerevisiae (SC) cells are single-celled eukaryotes and model organisms for studying cellular mechanisms including DNA damage and repair as well as systematic fungal infections. Additionally, *S. cerevisiae* shares the complex internal cell structure of animals without the high percentage of non-coding DNA that can confound research in higher eukaryotes. Three of the most extensively used antibiotic drugs for studying SC is amphotericin B (Amp B), rifampicin and fluconazole [3, 4]. The antibiotics target different cellular organs to elicit their antimicrobial and antifungal effects. For example, Amp B is membrane mediated thereby increasing the permeability of ions and small molecules by binding more strongly to ergosterol, the principal fungi sterol found in SC [5]. Rifampicin and fluconazole on the other hand have broad antibacterial and antifungal influence with rifampicin targeting different forms of mycobacteria by inhibiting DNA-dependent RNA polymerase activities, whereas fluconazole is used for a number of fungal infections including candidiasis as well as other fungal diseases. Since Amp B and rifampicin are redox mediators that can interact with eukaryotic cell membrane thereby increasing redox activity by creating pores or inhibiting the synthesis of ergosterol respectively, it will be interesting to compare their redox activity with fluconazole. These antifungal drugs were used as model drugs because they have been extensively used for various *in vitro* and *in vivo* studies of model cells.

Based on the background outlined, it is obvious that there are essentially two pathways (lipid-mediated and diffusion porins) through which both hydrophobic and hydrophilic plant metabolites or drugs elicit their potency at the cellular level. It is obvious that the degree of permeation of the cell membrane has a major impact on the redox activity. In addition, the presence of a hydrophobic drug within the complex architecture of the membrane, enhances easy access of small ions through pores which can be detected electrochemically. Non-membrane-mediated drugs diffuse freely through the membrane and may not necessarily destabilize the membrane architecture; thereby limiting ionic flow that can be captured by electrochemical detection techniques. In the current work, an entrapment strategy was developed using aluminosilicate minerals to selectively pool plant metabolites using different pH conditions and evaluating the polarization and depolarization of model cells using electrochemical sensing techniques. For membrane targeted bioactive compounds, the fluctuation in the redox signals will reveal important lead bioactive compounds for further investigation.

Several biopolymers and aluminosilicate minerals derived composite drug delivery carriers have been reported from several laboratories. For example halloysites natural tubules (HNTs) are aluminosilicate minerals composed of

different proportions of aluminum, silicon, hydrogen and oxygen often with the chemical formula $Al_2Si_2O_5(OH)_4.nH_2O$ [6, 7]. They are empty cylinders with widths of about 100 nanometers and consist of two structures; the anhydrous structure with an interlayer dispersing of approximately 7 Å and the hydrated structure with an augmented interlayer dividing of 10 Å, due to the presence of water in the lamellar spaces [8–10]. In each layer of the halloysite nanotube, the SiOH groups are found on the outer surface whiles the AlOH groups are situated on the inner surfaces making the outer and inner surfaces to have different charges [11–13]. The positive charge of the internal lumen is a consequence of protonation of the AlOH group at low pH whereas the SiOH groups have overall negative charge due to the coordination of the atoms. When the halloysites nanotubes are modified with biopolymer such as chitosan new functional materials with improved physicochemical properties are generated. The composite materials can serve as effective drug carriers.

The charge disparity of halloysites has also drawn interest from the research community whereby overall negatively charged proteins taken above their isoelectric points are mostly loaded into the positively charged nanotube lumen [14]. Therefore, in a pool of organic compounds, halloysites nanotubes can facilitate the formation of a transient bond between selected bioactive compounds and the AlOH or SiOH as a function of pH conditions and can be very effective as a nano drug carrier for different applications [15–19].

Traditional herbal medical practices continue to be part of the healthcare needs of the world especially residents of sub-Sahara Africa (sSA) [20–23]. However, the mechanism of action of the plant metabolites to illicit their potency continues to be a mystery due to the lack of standardized methods. Electrochemical detection of drugs interacting with most biological systems is an important strategy to understand cellular stresses that causes cell death [24–26]. Evidence emanating from previous findings, indicate that there are several membrane redox centers in most eukaryotic cells that can be targeted to monitor redox activities in the presence of certain drugs including plant metabolites [27–29].

The concept is investigated using extracts from *Dioclea reflexa (DR)* hook which belongs to the leguminous family. There are certain class of compounds in *Dioclea reflexa (DR)* that have clinical usefulness in both temperate and tropical regions [30–33]. Extract of *DR* seed has been shown to boost hematological parameters and antioxidant activities which protect the kidney and blood from oxidative and related injuries under acute and chronic toxicological challenges [30, 31, 33–37]. Also, the aqueous extract of the seeds produces 100% mortality in third stage mosquito larvae of *Aedes aegypti*. The seed is a potential food source which contains around 14% protein, 8% fats and 58% carbohydrates [32]. Though these metabolites continue to show promise in disease treatment, there is very limited data in the literature of the properties of single isolates and their medicinal relevance, albeit due to the difficulties in pursuing systematic separation of the complex mixtures in a single separation method. Thus, the current work describes the use of a simplified method to systematically pool bioactive compound mixtures from *DR* and test their inhibitory effects on breast (MCF-7) cancer cells and *Saccharomyces cerevisiae* (SC) cells. The rationale is that the larger surface area coupled with the differential polarity of the lumen and the surface of the halloysites nanotubes will be sufficient to bind selectively with the plant metabolites in the crude extracts of *DR*. The evidence of the entrapped species on the halloysites nanotubes was monitored using X-ray diffractometry (XRD) and Fourier transform infrared spectroscopy (FTIR) to determine the degree of aluminol (AlOH) and the siloxane (SiOH) groups modification since these two functional groups will be key sites for bioactive compounds interaction. pH-dependent eluted samples were then tested on breast (MCF-7)

cancer cell lines to investigate their inhibitory effects and the mechanism of inhibition were determined using cyclic voltammetry and flow cytometry analyses [38–41]. The results are reported here and show evidence of differential inhibitory effects of the bioactive compounds from the various pH conditions.

2. Methods

2.1 Extraction of bioactive compounds

Dioclea reflexa seeds were obtained from the District of Jaman North in the Brong Ahafo region of Ghana (7°57′1.8" North and 2°41′52.08" West). The content of the seeds was dried in the sun, and the cotyledon ground into powder using a laboratory mortar and pestle. The drugs used for the study were obtained from a Company (Sigma-Aldrich, Saint Louis, MO, USA). 5 gram of *Dioclea reflexa* Seed powder was dissolved in 30 mL of 70% ethanol. 5 mL of the supernatant was used for immobilization using 10, 50, 75, 100, 150 and 200 mg of HNTs. 200 mg gave the best entrapment. The loaded HNTs were released with a buffer with pH ranging from 4 to 9 and the contents vacuum dried followed by dissolution in 1% dimethyl sulfoxide (DMSO) to give a final concentration of 3.2 mg/mL.

2.2 Cell viability and electrochemical detection

The methods used for preparing stock solution of the drug are previously described [23]. Cells were treated with the drugs or the extracts to study the effects at prescribe time interval followed by estimating cell death. A correlation of cell death to electrochemical behavior was conducted using cyclic voltammetry under steady-state conditions as previously described [17].

3. Results

The stepwise procedure in this work probes the mechanism of action of standard drugs for treating microbial infections and the results compared to plant bioactive metabolites and chitosan nanocomposites. Two classes of standard drugs were used for comparison; anti-microbial drugs that include amphotericin B, rifampicin and fluconazole as well as cancer drugs which included curcumin and gossypol. Whereas the plant metabolites were derived from *Dioclea reflexa (DR)*. The therapeutic effect of nanocomposites were also tested. Chitosan nanocomposites were synthesized using chitosan as the base material and Tetraethyl orthosilicate (TEOS) as well as acetic acid as modifiers. Our hypothesis stipulated that drug candidate target membrane environment of cells leading to polarization or depolarization. The outcome in this case would enhance ionic mobility across membranes which could be captured through electrochemical detection as shown in **Figure 1**.

The various standardized drugs, plant metabolites as well as the synthesized nanocomposites used to investigate the electrochemical behaviour of the cells are displayed in **Table 1**. The cell lines and their sources are also indicated in **Table 2**. First, the electrochemical behaviour of *S. cerevisiae* cells was investigated using the standard antimicrobial drugs, amphotericin B, fluconazole and rifampicin. As shown in **Figure 2**, fluconazole and rifampicin exhibited very limited changes in the anodic peak potential (**Figure 2A** and **B**) compared to the amphotericin B doped *S. cerevisiae* cell lines (**Figure 2C**). The results obtained confirmed membrane polarization/depolarization behaviour of the *S. cerevisiae* due to the

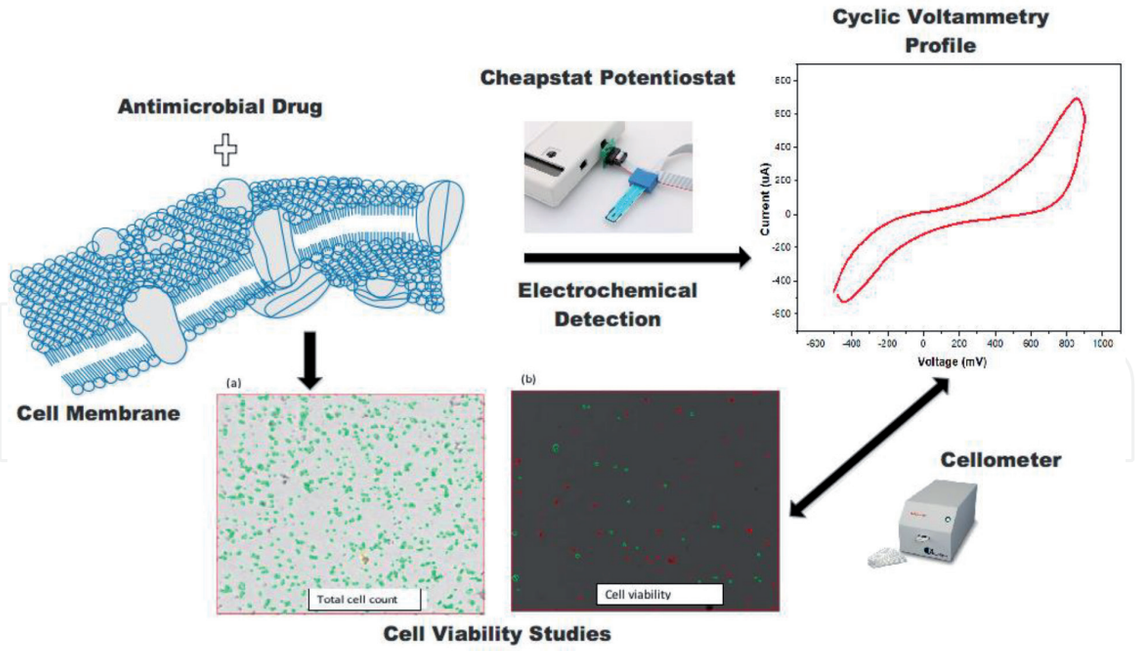

Figure 1.
Schematic illustration of the mechanism of drug interaction with biological membranes and how its electrochemical response (using a miniature electrode) correlates to cell viability as captured by the a cell counting device (Cellometer) [33].

Compound	Source	Structure
Fluconazole	Human milk	Triazole
Rifampicin	Streptomyces	Polyketide
Amphotericin	Streptomyces nodosus	Polyene with seven adjoining trans double bonds
Gossypol	Cotton plant	Natural phenol
Curcumin	*Curcuma longa* plants	Beta-diketone
Chitosan nanocomposite*,1	Syntheisized from chitosan and Tetraethyl orthosilicate (TEOS)	Chitosan modified with TEOS in different sequence (no known structures)
Dioclea reflexa Extract*,2	Seed	pH dependent elution of plant metabolites from halloysites nanotubes (no known structures)

**Extracted or synthesized and unpurified.*
[1]The following composites were tested: CT; chitosan modified by TEOS followed by acetic acid, CC; chitosan modified with TEOS/acetic acid mixture, CA; chitosan modified with acetic acid, and CT, chitosan modified with TEOS.
[2]Plant metabolites obtained from pH ~4.1–9.6.

Table 1.
List of compounds used for the study.

presence of amphotericin B leading to increase ionic mobility. Fluconazole and rifampicin which are not directly linked to membrane destabilization as the former are mainly responsible for RNA synthesis inhibition and rifampicin being responsible for inhibition of ergosterol synthesis.

A correlation between cell death and electrochemical response was established using *S. cerevisiae* cell lines and MTT assay detection. Although the result indicated cell death in the presence of the antimicrobial drugs, Amphotericin B exhibited

Cell lines	Source	Redox behavior	Cell viability
Prostate cancer (PC3)	ATCC (Manassas, VA)	Cyclic Voltammetry	Trypan blue and MTT assays
Breast (MCF-7) cancer	ATCC (Manassas, VA)	Cyclic Voltammetry	Trypan blue and MTT assays
S. cerevisiae	(ATCC/LGC Standards, Teddington, UK)	Cyclic Voltammetry	MTT assay

Table 2.
Cell lines and Detection methods used for Investigating membrane mediated effects.

enhanced cell death probably due to the high degree of membrane permeability of ions as indicated in the voltammograms in **Figure 2C**.

To test the electrochemical behaviour and redox activity of the *Dioclea reflexa* extracts, cyclic voltammetry analysis was conducted using interdigitated gold electrodes (IDEs), (Metrohm, DropSens). The current from the quasi-reversible oxidation curve was plotted against concentration of the plant metabolites.
Figure 3A (*control, black*) indicated insignificant redox mechanism, however, the extracts showed quasi-reversible oxidation values ranging from 0.25 to 0.70 mA at a scan rate of 10 mV/s. The water extract (SWE) had higher redox potential compared to those of the ethanol extract (SEE). The water extract (SWE) *(red)* demonstrated the most cell death followed by methanol extract (SME) (*purple*) and ethanol extract (SEE) (*blue*) respectively with the control cells *(black*) exhibiting the least cell death at higher concentrations and extended incubation time periods. *SWE* (*red*) revealed cell death of about 57%, whereas *SME (purple), SEE (blue*) recorded about 31 and 22% respectively at the same concentration. It was concluded that the extracts caused membrane porosity to initiate reactive oxygen species release leading to cell death.

Another important strategy in plant phytochemical studies is to develop local immobilization materials that can capture bioactive plant metabolites and release the cargo steadily onto diseased cells. Optimization parameters were developed to capture plant metabolites from *Dioclea Reflexa (DR)* seed extracts on halloysites nanotubes (HNTs). An encapsulating capacity of 13% was obtained when approximately 5 g of *DR* extracts was immobilized onto about 1 g of HNTs. Evidence of plant metabolites entrapment was monitored with FTIR and X-ray diffraction methods. As shown in **Figure 4A**, changes in the FTIR signatures peak intensities of the halloysite nanotubes (HNTs) revealed all the functional groups present in the empty halloysites nanotubes (black). The inner Al-OH and outer Si-OH groups have characteristic stretching peaks at 3624 and 3691 cm^{-1}, respectively. Bending vibrations of Al-OH and Si-O revealed absorption peaks at 907 cm^{-1}. In addition, the uneven stretching vibrations of the Si-O bond gives a strong absorption peak at 1005 cm^{-1}. There was a significant reduction of the transmission peak after immobilization of the *DR* extracts on the halloysites nanotubes (blue) and this indicated a modification of the nanotubes with the plant metabolites. Following the release of the bioactive compounds from the nanotubes, the transmission peaks reverted to the original peaks of the empty nanotubes (red). Similarly, **Figure 4B**, showed the characteristics 2θ° peak positions of the nanotubes which occurs at 11.7, 20.5, 24.8, 37.5, 43.3 and 64.4° (red). After immobilization of the *DR* extract on the nanotubes, there was dramatic reduction of peak intensities at the same 2θ° positions and that indicated chemical modifications of the nanotubes by the bioactive constituents in *DR* extract (black). The bioactive constituents were eluted with 70% ethanol and resulted in the reversal of the nanotubes peaks as shown in **Figure 4B** (blue). The characteristic peak intensities reverted to those observed in the control.

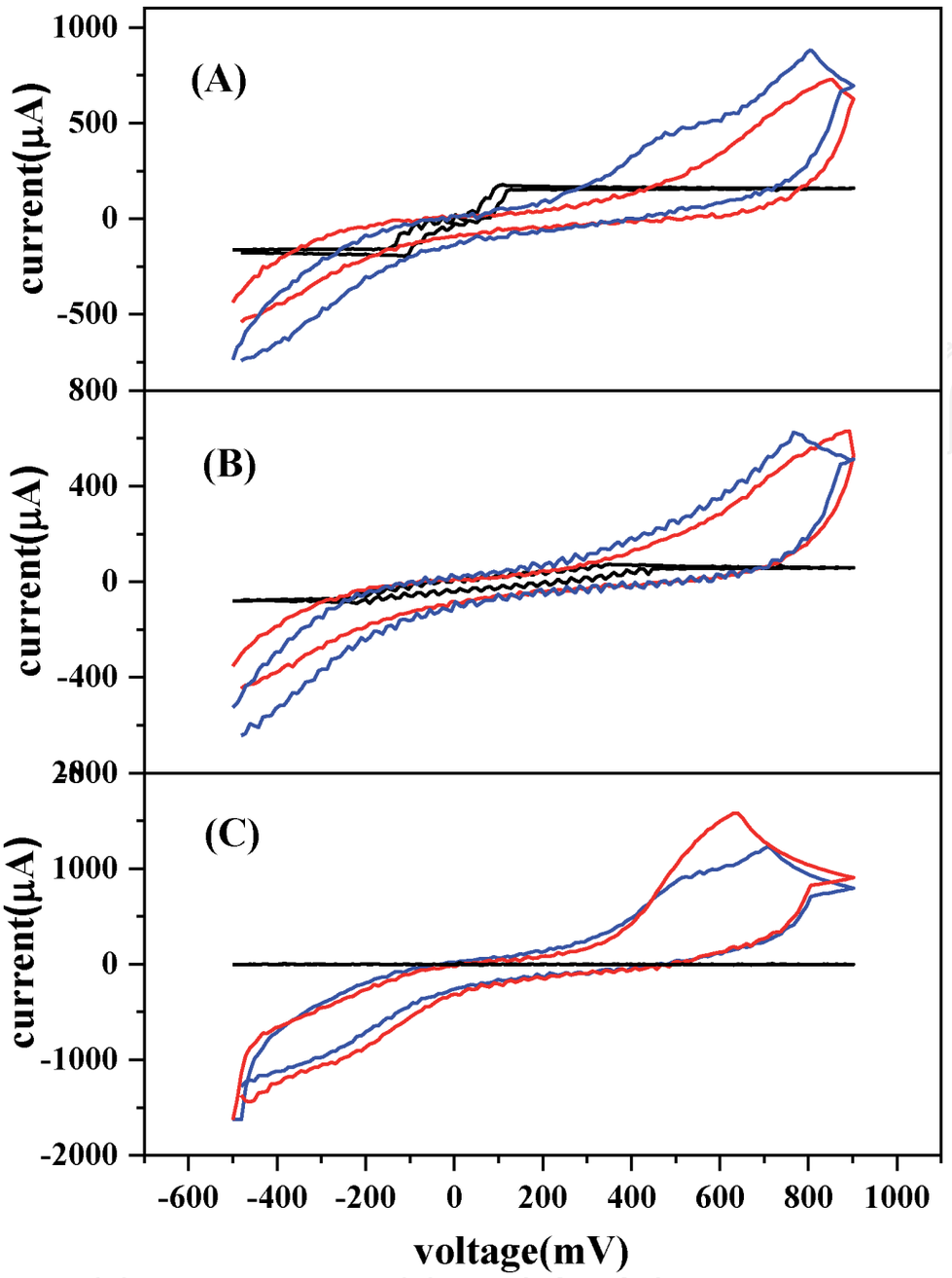

Figure 2.
Cyclic voltammetry response of S. Cerevisiae *in the presence of (A) rifampicin, (B) fluconazole, and (C) amphotericin B on IDE electrode (Colour code: black, antibiotic; red, cells and blue, cell + antibiotic).*

The antiproliferative activity of the crude extracts did not reveal significant inhibitory effects on breast (MCF-7) cancer cells, however, the pH-dependent eluted metabolites revealed that the acidic pH samples exhibited profound antiproliferative effects on the cancer cells compared to the basic pH metabolites using both trypan blue dye exclusion assay and MTT viability test as shown in **Table 3**. pH ~ 5.2 demonstrated IC_{50} of 0.8 mg and a cyclic voltammetry oxidation peak potential and current of 234 mV and 0.45 μA respectively indicating membrane polarization/depolarization of the cancer cells as shown in **Figure 5**. It was confirmed through fluorescence-activated cell sorting (FACS) studies that the plant metabolites influenced breast cancer apoptotic signaling pathways of cell death as shown in **Figure 6**. The studies proved that plant metabolites could be captured using simplified screening procedures for rapid drug discovery purposes. Such procedures, however, would require the integration of affordable analytical tools to isolate individual metabolites

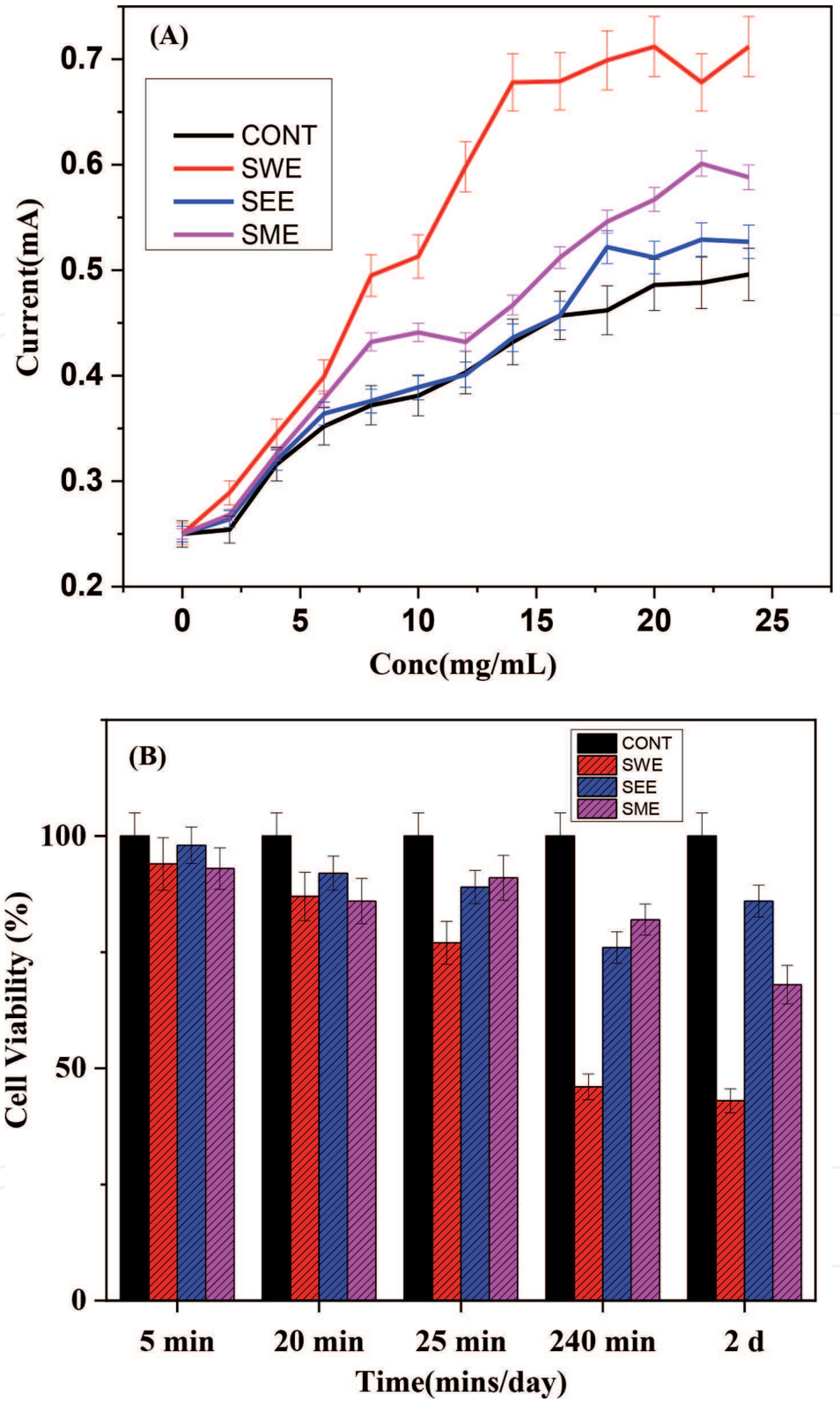

Figure 3.
(A) A graph of current versus concentration of plant extracts revealed the water extract generated highest current, followed by the methanol extract, ethanol extract and the control cells in that order. (B) Investigating cell death as a function time. The water extract revealed significant cell death (red) *after 2 days of incubation, followed by the methanol extract, ethanol extract and control in that order.*

for testing. Our approach could be an important strategy to create plant metabolite database based on pH values.

Biopolymers such as chitosan, gelatin and cellulose have been used with different additives in order to modify their surfaces for biomedical application. In the

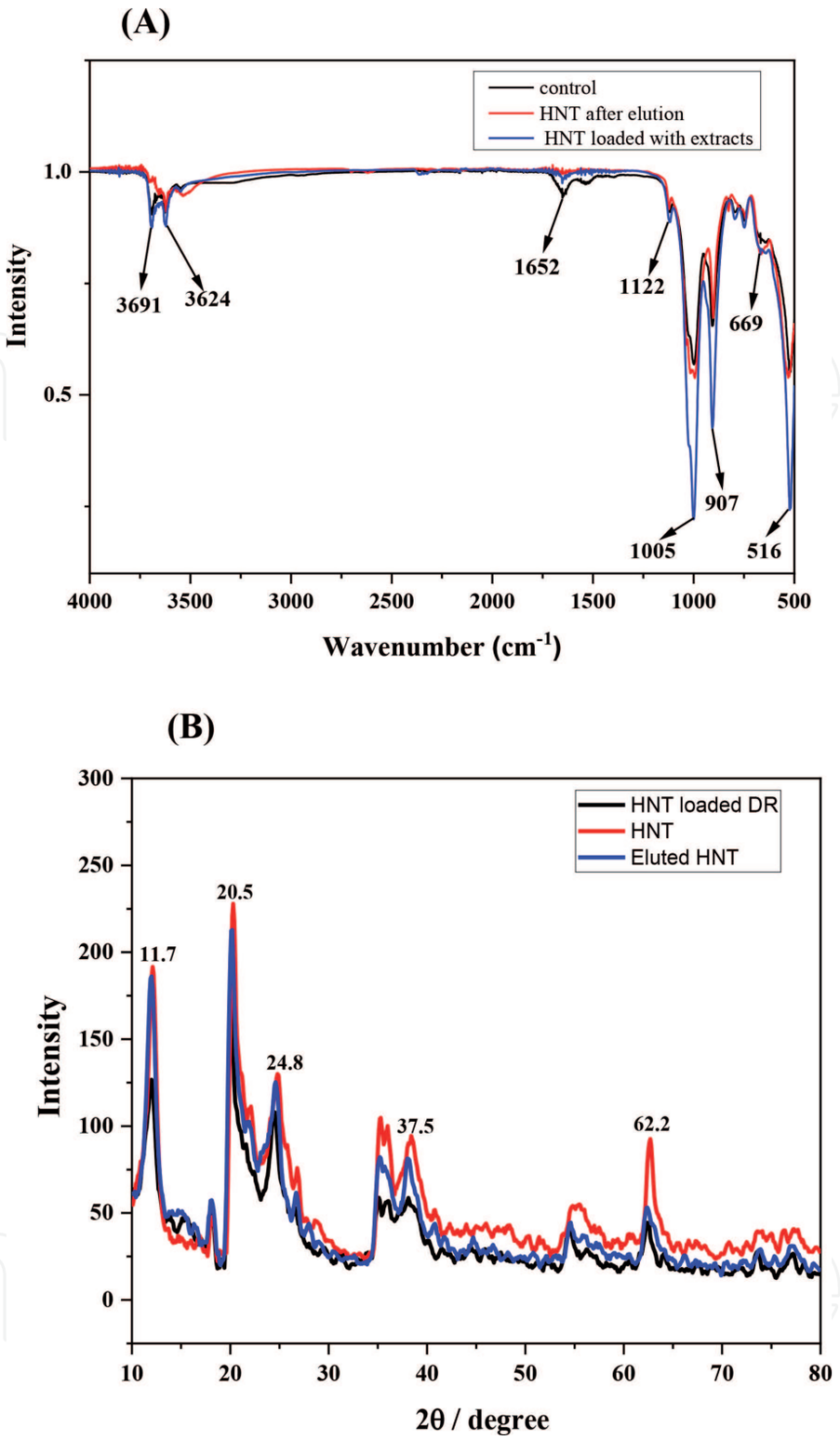

Figure 4.
(A) Characterization of HNTs and loaded HNTs with plant metabolites using FTIR techniques. Reduction in IR transmittance indicates OH on the HNTs are functionalized by at least one bioactive. (B) XRD spectra of the entrapped metabolites showing the signature peaks at two theta position of HNTs and loaded HNTs with plant metabolites. Intensity has inverse relationship to surface area. Larger area due to immobilization will cause a decrease peak intensity.

next paragraph, a description of the synthesis of chitosan nanocomposites using tetraorthosilicate (TEOS) and acetic acid (AA) to study their influence on prostate cancer (PC3) cell lines would be described. The particles synthesized for the study include *SC*; chitosan modified by TEOS followed by acetic acid, *CC*; chitosan modified with TEOS/acetic acid mixture, CA; chitosan modified with acetic acid, and

	Normalized	
Sample/Dry extract	**IC_{50} (mg)**	**R squared value**
Water	33.3	0.959
Ethanol	1.6	0.991
pH 4.1	1.4	0.967
pH 5.2	0.8	0.998
pH 6.4	1.6	0.975
pH 7.4	1.9	0.983
pH 8.1	2.3	0.948
pH 9.6	3.1	0.994

Table 3.
Experimental IC_{50} values of the extracts eluted at different pH conditions all measured in milligram quantities of the seed extract.

CT, chitosan modified with TEOS. As shown in **Figure 7A** and **B**, The electrochemical response of normal cells and prostate cancer cells (PC3) when treated with the particles revealed the latter showed modest response whereas the PC3 cells anodic peak currents changed dramatically with the treatment with the nanocomposites especially the *SC* nanoparticles. The cell viability studies revealed a corresponding decrease in cell viability as measured by a cell counting device. The normal cells again showed no significant difference in cell viability after 24–48 hours of cell growth as shown in **Figure 7C** and **D**. The results were compared with a standard drug used to treat cancers, gossypol (GP).

4. Discussion

In the current work, a systematic approach was undertaken to carefully investigate standard drugs, plant metabolites and chitosan nanoparticles on the electrochemical behaviour of selected cell lines and also to correlate their electrochemistry to cell viability.

It is becoming evident that taking advantage of the numerous redox mediators, scientists could develop biosensors from many biological systems. For example, *S. Cerevisiae* has several Redox centers which could be exploited using hydrophilic/hydrophobic molecules as extensively discussed previously by *Rawson et al.* [1, 2]. The fact that Electrochemical behavior could be monitored using membrane targeted drugs [42–45], opens avenue for future development of a biosensor for identifying potential drug candidate from plant sources. For example, the famous antifungal drug, Amp B has been used to treat fungal infection effectively and its mechanism of action has been well characterized [46, 47]. It is established that the antifungal drug binds to ergosterol in the cell membrane to enhance leakage of ions leading to depolarization of the membrane [48]. Increase in ions leakage across membrane could ultimately increase the oxidation potential across membranes. Such source of ions can be detected through electrochemical techniques as already observed in our studies as well as studies from other groups [2].

The fact that Amp B and the plant extracts behave similarly on *S. cerevisiae* cell viability supported a general claim that Amp B and the plant extracts exhibited a common mechanism leading to cell death. Hence, due to the quasi-reversible oxidation process observed in the anodic response, it was concluded that membrane

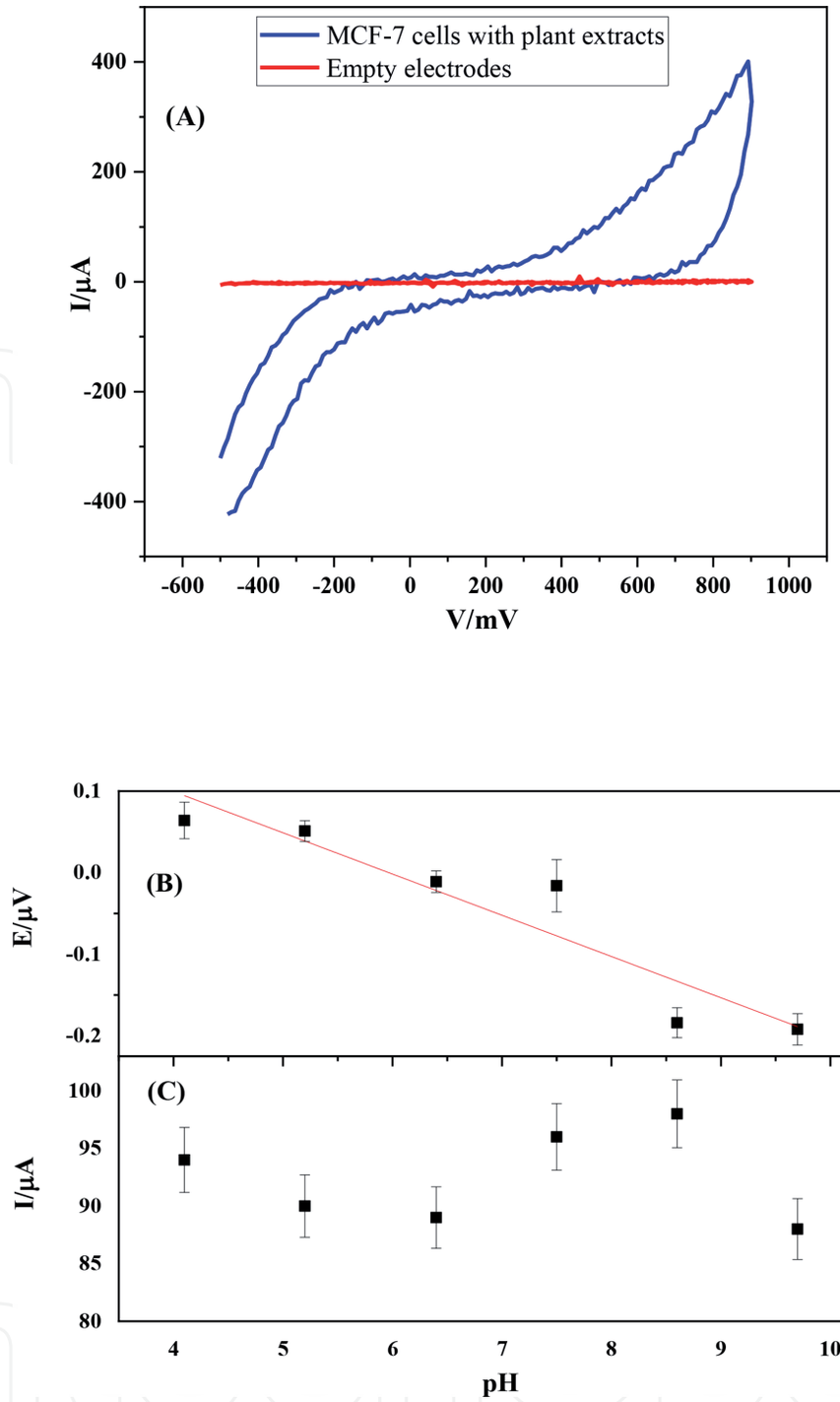

Figure 5.
(A) Effects of the bioactive compounds from the pH ~5.2 on the depolarization potential of the MCF-7 cells. (B) The influence of the voltage on current of the MCF-7 cells as a function of pH. The cyclic voltammogram measurements conditions were: Scanning from 690 mV to 970 mV at a scan rate of 10 mV s^{-1}. MCF-7 cancer cell viability studies of the bioactive compounds extracted at pH ~ 5.2 at cell concentration of 1 x10^6 cells/well [submitted results for publication, Scientific Reports].

polarization/depolarization leading to ionic leakage might have been the mechanism through which one or more of the organic bioactive molecules illicit their action. This correlation had highlighted an important opportunity that could be further exploited for identifying bioactive plant metabolites in the natural product field.

We used pH dependent elution of the *DR* bioactive compounds from the halloysites nanotubes to further validate the activity of the captured metabolites on electrochemical behaviour and cell death. Halloysite nanotubes have SiOH and AlOH

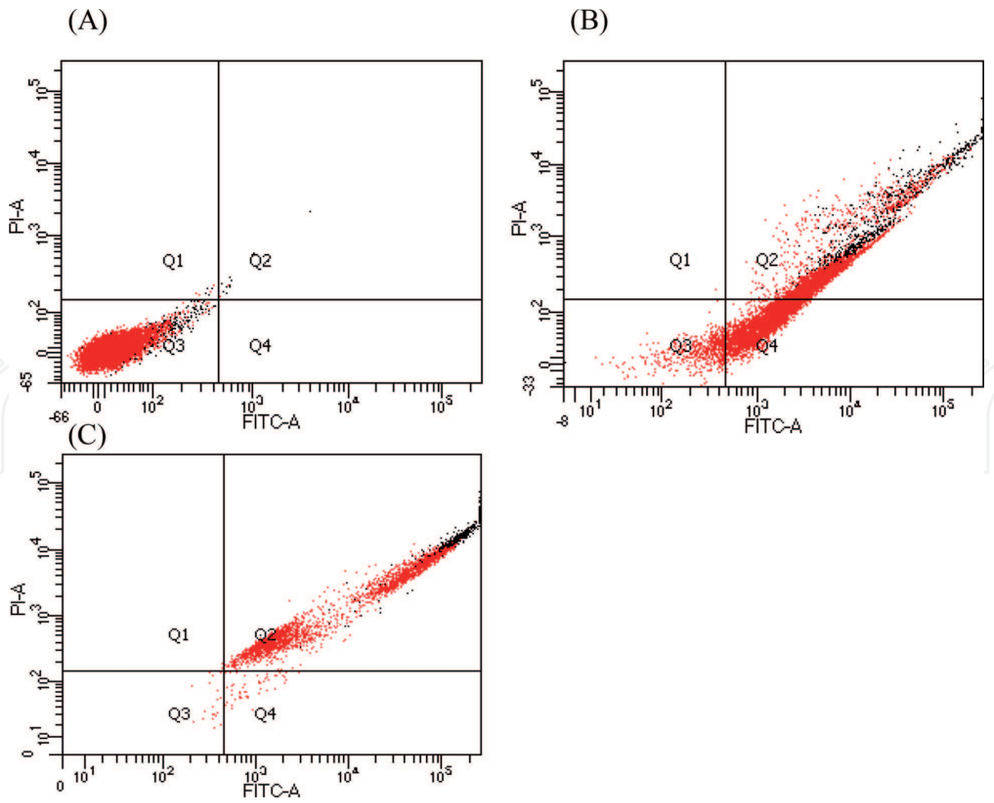

Figure 6.
Fluorescence activated cell sorting (FACS) analysis of the inhibitory effects of breast (MCF-7) cancer cells using bioactive extracts at pH ~ 5.2 (best IC_{50} concentration). The results were compared to the inhibitory effects of a Commercially available cancer drug, cucurmin. (A) untreated cells, (B) cells treated with extract and (C) cells treated with curcumin all at cell concentration of 1 x10^6 cells/well.

groups which are found on the outer surface and the inner surface making the outer and inner surfaces to have different charges respectively [10, 49, 50]. Thus, depending on the pH conditions, aluminol (AlOH) and the siloxane (SiOH) groups could either be protonated or deprotonated leading to different affinities towards certain macromolecules and organic compounds. Our hypothesis was that partially positive metabolites will be weakly attracted to SiOH groups whereas negatively charged metabolites would prefer the latter. The pH dependent release of the metabolites from the HNT were not statistically different after determining the amount in milligram quantities and expressing the entrapment efficiency as a percentage value. However, when tested against the breast (MCF-7) cancer cell lines, the acidic pH elution demonstrated significant anti-proliferative activity against the cancer cell lines compared to the basic pH metabolites. The most profound activity was found in the pH ~ 5.2 which was supported by IC_{50} calculated values.

Depolarization is an indicator of mitochondrial dysfunction in most cancer cells and therefore investigating polarization and depolarization could inform the mechanism of cell death [51]. In this work, Cyclic voltammetry measurements were used to probe the extent of polarization and depolarization by relating the voltage to current surge using electrochemical methods. The results revealed that the metabolites exhibited quasi-reversible redox behavior and concentration dependent reduction in the applied voltage [52]. The currents also showed a triangular modulation with a rise in oxidation current at lower pH, followed by another rise beyond acidic pH and further reduction to the strongly basic pH. Metabolites from the pH ~ 5.2 extract required a higher voltage application to generate the minimum amount of current in the cells indicating cell membrane polarization in the presence of the metabolite was achieved. The extracts from pH ~ 7.4 and pH ~ 8.1, even though gave higher IC_{50} values, the voltage required to initiate cell depolarization

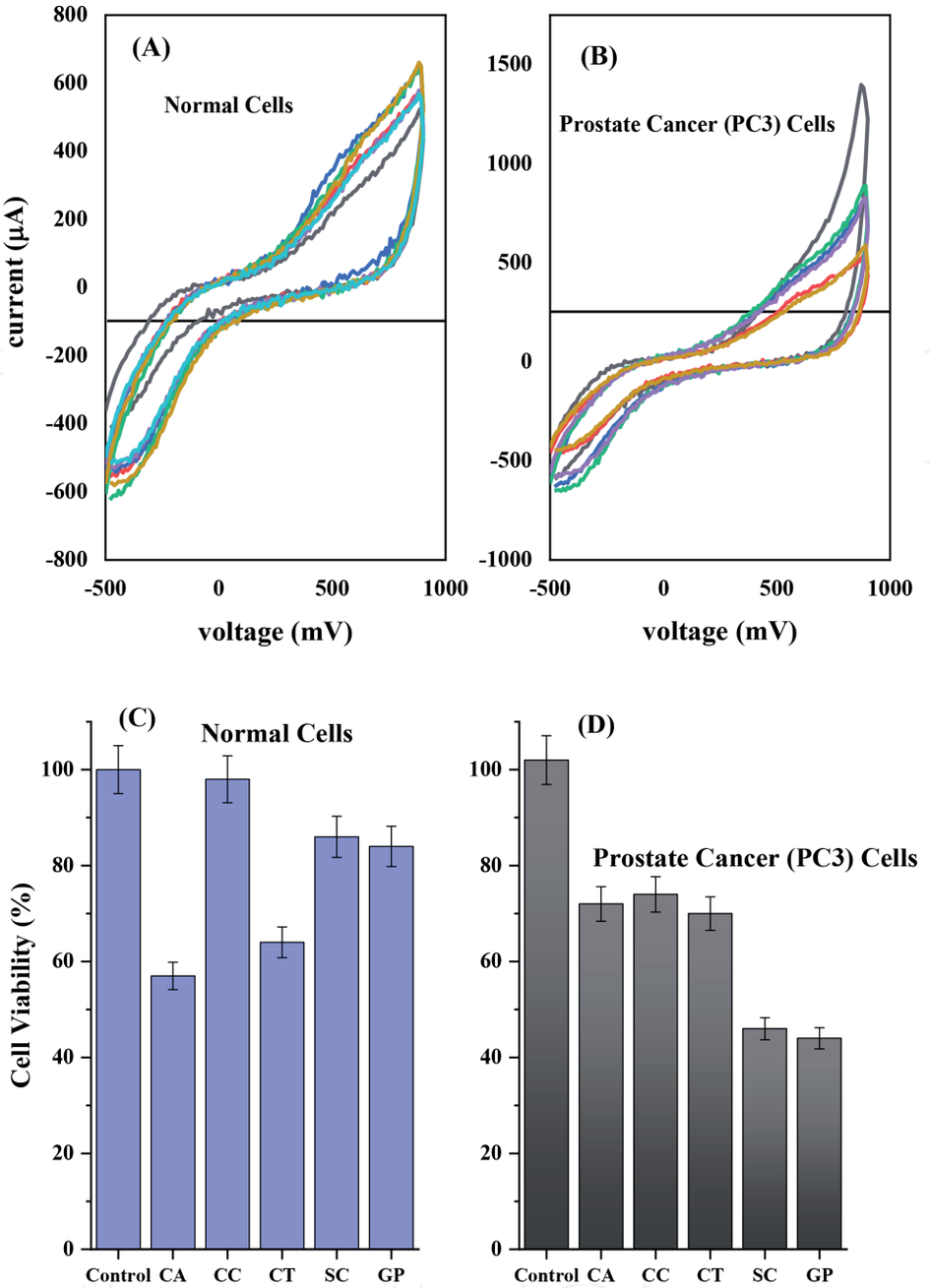

Figure 7.
Correlation electrochemical response of the (A) normal cells and (B) prostate cancer cells (PC3) as a function of chitosan composite treatment. SC*; chitosan modified by TEOS followed by acetic acid,* CC*; chitosan modified with TEOS/acetic acid mixture,* CA*; chitosan modified with acetic acid, and* CT*, chitosan modified with TEOS The corresponding cell viability as measured by the cell counting device. (C) normal cells and (D) cancer cells.*

in the cells was at a minimum as indicated in the maximum current generation. The results highlighted that the metabolites could cause cell death through a polarization/depolarization mechanism as documented by other researchers in the literature [53]. Flow cytometer-based analysis showed that the metabolites showed dose-dependent apoptosis of MCF-7 cells. It was noted that exposure of 2 $mgmL^{-1}$ concentrations of the metabolites led to greater than two-fold increase in apoptosis in comparison to the untreated cells. Curcumin is a well-known polyphenol and widely used for its anti-oxidative and anti-cancerous application. Curcumin effects on the breast cancer cells were also investigated and compared with the result from the metabolites. It was observed that curcumin improved cell death significantly without going through the apoptotic phase indicating synergistic effect could be

developed when both metabolites and curcumin are used to treat cancer. Finally, the chitosan nanoparticles also demonstrated that membrane polarization and depolarization could also be used to monitor particle permeation into cell membrane to induce varied cell behaviour.

5. Conclusion

In conclusion, electrochemical behaviour of cells in the presence of plant metabolites if carefully pursued could help establish a database with fundamental information on herbal medicine isolation and characterization to serve the scientific community in future studies of herbal medicine. It is also simple, robust and required a specific protocol that could be adopted for understanding the medicinal plants behaviour for easy characterization. It has to be emphasized that further work needs to be pursued to understand the mechanism of these membrane targeted behaviours.

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