SOMATO PUBLICATIONS

Journal of Clinical Nutrition and Food Science

Research Article

Cytotoxic Potentials of *Clerodendrum Volubile* against Prostate Cancer Cells and Its Possible Proteomic Targets

Saheed Afolabi[1], Olufunke Olorundare[1], Gideon Gyebi[3], Deeba N Syed[2]*, Hasan Mukhtar[2] Ralph Albrecht[4] and Mamoru Koketsu[5]

[1]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara, Nigeria

[2]Department of Dermatology, University of Wisconsin, Madison, WI 53706, USA

[3]Department of Biochemistry, University of Ilorin, Ilorin, Kwara, Nigeria

[4]Department of Animal Sciences, 1046 Animal sciences building, University of Wisconsin, Madison, USA

[5]Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University, Gifu, Japan

***Address for Correspondence:** Syed Deeba N, Department of Dermatology, University of Wisconsin, Madison, WI 53706, USA, E-mail: dsyed@dermatology.wisc.edu

Received: 19 August 2019; **Accepted:** 13 September 2019; **Published:** 14 September 2019

Citation of this article: Afolabi, S., Olorundare, O., Gyebi, G., Syed, DN., Mukhtar, H., Albrecht, R., et al. (2019) Cytotoxic Potentials of Clerodendrum Volubile against Prostate Cancer Cells and Its Possible Proteomic Targets. J Clin Nutr Food Sci, 2(2): 046-053.

ABSTRACT

The plant *Clerodendrum volubile* consumed by the Niger/Delta people of Nigeria has additional therapeutic applications for diabetes, ulcer, arthritis, rheumatism, dropsy, etc. The present study was conducted on the methanol extracts from *Clerodendrum volubile* leaves (CVE), with the aim of exploring its cytotoxic activity against prostate cancer (PCa) cells and delineate its potential mechanistic targets. CVE inhibited cell viability and suppressed clonogenic potential of PCa cells as assessed by MTT and colony assays. Western blot analysis of CVE-treated cells revealed concentration-dependent cleavage of PARP and Caspase 3. Flow cytometric data validated apoptosis as the primary mechanism of CVE-induced cell death with significant increase in the number of apoptotic cells subsequent to treatment. Furthermore, CVE-treated cells demonstrated increased levels of cyclin- dependent kinase inhibitor p21, suggesting modulation of cell cycle machinery. Profiling of CVE-treated PCa cells, utilizing a LC-MS/MS based quantitative proteomic strategy, yielded a data set of 16 significantly modulated proteins. Other than three proteins, ALBU, ROA3 and HNRPC that were downregulated [1.7; 1.7 and 2.5 fold, respectively], 13 proteins including TALDO, SYWC and FUMH with specific roles in the pentose phosphate pathway and tricarboxylic acid cycle were significantly upregulated [2.4; 1.9 and 1.8 fold, respectively] in CVE-treated PCa cells. Our studies provide insight into plausible mechanism(s) through which CVE exerted its growth inhibitory effect against PCa cells. Further studies are warranted to characterize the effect on functional pathways modulated by CVE with special focus on glucose metabolism.

Keywords: Chemoprevention, Prostate cancer, *Clerodendrum volubile*, Proteomics, Apoptosis

Introduction

The International Agency for Research on Cancer reported prostate cancer (PCa) as the fourth leading malignancy globally with an incidence rate of 7.9% [1]. Recently, PCa has ranked high (26%) on the percentage of newly diagnosed cancer cases in the United States and 9% of cancer- related deaths amongst men [2]. Thus, it poses a huge public health challenge [3]. Sedentary lifestyle such as lack of physical activity and low consumption of fruit and vegetables and high intake of tobacco and alcoholic beverages have been suggested as predisposing factors to its occurrence [4]. The currently available treatment options to combat PCa are far from satisfactory given the associated side effects coupled with development of drug resistance. It is imperative to discover newer therapeutic approaches with higher efficacy to reduce the incidence of the disease associated mortality and improve survival [5]. An attractive approach therefore would be to identify plant-based agents that can exert cytotoxic effect against PCa

cells, augment chemotherapy, show efficacy against drug-resistant cases and exert fewer side effects.

Herbal remediation as a main therapeutic approach or in combination with already established conventional medicines remains a viable tool in the provision of adequate and robust health care [6-8]. Several factors including affordability, accessibility and easy availability make this an attractive option in both developed and developing countries [9,10]. In addition, the use of phytomedicinal plants and nutraceuticals as chemopreventive/therapeutic agents is receiving acceptability due to scientific reports confirming their efficacy in preclinical and clinical models [11].

The field of traditional, complementary and alternative medicines is opening new leads for drug discovery and combating resistance to several chemotherapeutic agent [12]. In accordance, there is increasing interest in the isolation of the active constituents, toxicology, and mechanistic studies to delineate novel therapeutic targets [13]. *Clerodendrum volubile* is a food condiment popular amongst the Niger/Delta people of Nigeria with ethnomedicinal relevance [14]. Locally called "Obenetete", it is used in the management of ulcer, rheumatism, diabetes mellitus, etc. (15). The goal of this study was to evaluate the cytotoxic activity of *Clerodendrum volubile* extract (CVE), delineate its potential mechanism(s) of cytotoxicity and identify its proteomic targets.

Materials and Methods

Plant material

Leaves of *Clerodendrum volubile* were collected from a farm settlement in Edo State, Nigeria between August 2015 and September 2016. The plant was identified and authenticated by Ajayi Bolu, a plant botanist from the Department of Botany, University of Ilorin, Ilorin, Nigeria and a voucher specimen number: UILH/01/019/1254 was deposited in the University Herbarium.

Reagents and antibodies

All primary antibodies were obtained from Cell Signaling Technology. Anti-mouse and anti-rabbit secondary antibody horseradish per-oxidase conjugates were obtained from Amersham Pharmacia Life Sciences. The Bio-Rad DC Protein Assay Kit was purchased from Bio-Rad, CA. Novex precast Tris-Glycine gels were obtained from Invitrogen. BD Annexin V FITC Assay kit was obtained from BD Biosciences, USA.

Cell lines

PC3, DU145 and C4-2 human prostrate carcinoma cells were purchased from ATCC (American type culture collection) and grown in RPMI 1640 (Life Technologies, NY) supplemented with 10% FBS and 1% penicillin/streptomycin, with 5% CO2, at 37°C. CVE dissolved in DMSO was used for the treatment of cells. Cells at a confluency of ~70% were treated with CVE at 10– 100µg/ml for 24h in complete cell medium where the final concentration of DMSO used for each treatment was less than 0.1% (v/v).

Plant extraction

Leaves of Clerodendrum volubile were air-dried to constant weight, powdered and macerated in methanol at room temperature for 7 days. Residue was re-macerated in methanol twice to get maximum plant yield. The filtrate from the repeated maceration was concentrated in vacuo to give a percentage yield of 5.04%.

Cell proliferation assay

MTT assay (3-4, 5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide) was employed to study the effect of CVE on the proliferative potential of PC3, DU145, C4-2 and PC-3M-luc-6 PCa cell lines. Cells were plated (1×10^4 cells per well) in 1ml of complete culture medium containing 10–200µg/ml concentrations of CVE in 24-well microtiter plates. After incubation for 24 and 48 hrs, time-points in a humidified incubator at 37°C, 200µl of MTT (5mg/ml: 1XPBS) was introduced into each well and incubated for two hours, with subsequent addition of 200µl of DMSO. The plates were then centrifuged (1800×g for 5min at 4°C) and absorbance taken at 540nm on a microplate reader. The effect of CVE on cell proliferation was calculated as % cell viability.

Colony formation assay

Colony formation assay was conducted to determine the long-term effect of CVE treatment on PCa cells. Both DU145 and PC3 cells were treated with CVE (40 & 80µg/ml) in RPMI-1640 complete medium. Following treatment, 5000 cells/well were re-plated in triplicate on a 6-well tissue culture plate in 5% CO_2 at 37°C for 8 days, with growth media being replaced with/without CVE every 2 days. The cells were then stained with 0.5% crystal violet (in methanol: H_2O; 1:1) and pictures were taken using a digital camera. The colony intensity was measured using the imageJ software.

Apoptosis monitoring by flow cytometry

Employing the methods described by Shabbir et al. [16], DU145 cells treated with CVE (40 & 80µg/ml: 24h) in complete medium were trypsinized and fixed in 1% Paraformaldehyde: 1X PBS for an hour, washed twice with cold PBS and suspended in chilled 70% ethanol. Next day, cells were centrifuged for 5min at 1000 rpm, and the pellet obtained was washed twice with cold PBS to remove ethanol. The cells were labeled with FITC using the Apo-Direct Kit (BD Pharmagen, CA) as per manufacturer's protocol. Analysis was performed with a FACScan (Becton Dickinson, NJ).

Protein extraction and western blot analysis

After treatment of DU145 and C4-2 cells with CVE (40, 80 and 100µg/ml), ice-cold lysis buffer (Cell Signaling Technology, Danvers, MA) was added to the cells along with protease inhibitors (Calbiochem, Germany). Cells were homogenized by passing through a 23gauge needle, centrifuged and the protein concentration of the supernatant was quantified using the BCA protein assay kit. 40-60µg of protein were resolved on 8–12% poly acrylamide gels, transferred on to a nitrocellulose membrane, probed with appropriate primary and secondary antibodies, and detected by chemiluminescence autoradiography.

Sample preparation and LC/MS/MS analysis

For quantitative proteomics analysis, the protocol designed by Singh et al. [17] was used with slight modification. PC3 cells were treated with vehicle (culture medium) or CVE (80µg/ml) for 24 h in triplicate. The experiment was repeated to yield 6 replicates. Treated cells were collected by trypsin digestion followed by centrifugation and washing with PBS to obtain cell pellets, which was subjected to nano-LC/MS/MS analysis, performed at the School of Pharmacy, UW-Madison. In brief, proteins were extracted from the frozen cell pellets after addition of 0.3 ml ice-cold PBS and passing cells through a 23gauge needle 10–15 times. Cell lysates were then cleared by centrifugation at 10,000 g for 10 min at 4°C. Micro BCA (Thermo

Fisher Scientific / Pierce) then determined protein concentration of the extracts. Sample protein (20 µg) was digested with 1 µg sequencing grade trypsin (Promega Corp.). Following an overnight digestion, samples were prepared for LC/ MS/MS by C18 Zip-Tip purification according to the manufacturer's protocol (Millipore Inc.). Samples were then suspended in water with 0.1% formic acid (v/v) and subjected to nano-LC/MS/MS.

For LC/MS/MS, the samples were analyzed by injecting 1 µg of the digest onto a reverse phase BEH C18 column (100 µm x 100 mm), with 1.7 µm, 300 Å pore particles size (Waters Corp. Milford) using a Waters nano ACQUITY chromatography system. Peptides were eluted from the column using a 180 min increasing organic gradient. Solvent A was water/0.1% formic acid (v/v), while solvent B was acetonitrile/0.1% formic acid (v/v). The gradient started at 3% B and increased with a linear gradient to 35% B at 130 min. At 140 min., the gradient increased to 95% B and held for 10 min. At 160 min the gradient returned to 3% to re-equilibrate the column for the next injection. Data-dependent MS/MS on a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific Inc.) analyzed peptides eluting from the column. A top 15 method was used to acquire data. The instrument settings were as follows: the resolution was set to 70,000, the AGC target was set to 106 counts, the scan range was from 300–2000 m/z, the MS scan was recorded in profile while the MS/MS was recorded in centroid mode, dynamic exclusion was set to 25 seconds.

Data processing and protein identification by human database search

Following LC/MS/MS acquisition, the data were searched against the Swiss-Prot human proteome database with decoy using Sequest HT search engine under the Proteome Discoverer 1.4 software (Thermo Fisher Scientific Inc.). Proteins were identified at a false discovery cut off < 1%. Following protein identification, the LC/MS/MS data were aligned using Chromalign software. Quantitation of peptides eluted between 30 and 130 min were performed on the processed data using SIEVE 2.1 (Thermo Fisher Scientific Inc.).

Pathway analysis

To understand pathways modulated by CVE, a list of differentially expressed (> 1.7 fold) was compiled. These proteins were categorized according to their Gene ontology (GO) descriptions using information from the GO database and PANTHER (Protein Analysis Through Evolutionary Relationships; http://www.pantherdb.org/) classification systems. The proteins were classified based on their molecular functions, biological processes and protein classes. The canonical pathways, disease/function pathways and protein-protein interactions were analyzed using Ingenuity Pathway Analysis Software (IPA, Ingenuity Systems, http://www.ingenuity.com) (Qiagen). The predicted protein-protein interaction networks and canonical pathways were generated using inputs of gene identifiers, log2 fold-changes and *p*-values between control and treated group comparisons.

Statistical analysis

All statistical analysis was carried out with GraphPad prism 6 (San Diego, CA), using student T- test, p values <0.05 were considered statistically significant.

Results

CVE exerted a deleterious effect on short- and long-term PCa cell viability

To examine the effect of CVE on cell viability, MTT assay was performed on CVE-treated androgen-independent (PC3 and DU-145). A concentration-dependent inhibition of growth and viability in CVE treated (10- 100µg/ml for 24h and 48h) PCa cells was observed. Time course analysis indicated a further decrease in cell viability at 48 h. As shown in Figure 1, the IC50 values of CVE-treated were 125.0 & 72.2µg/ml for PC3 and 88.2 & 58.1µg/ml for DU-145 cells. On a long-term scale as assessed by clonogenic survival, CVE (40 and 80µg/ml) mediated a significant concentration-dependent inhibition of colony formation relative to untreated controls (Figure 2) in DU-145 and PC3 cells, treated for seven days.

CVE increased the fraction of apoptotic DNA in PCa cells and induced Caspase 3 mediated apoptosis with a concomitant activation of CDKI-p21

Apoptosis detection and quantification was assessed to understand the mechanism of observed cell death. Using Annexin

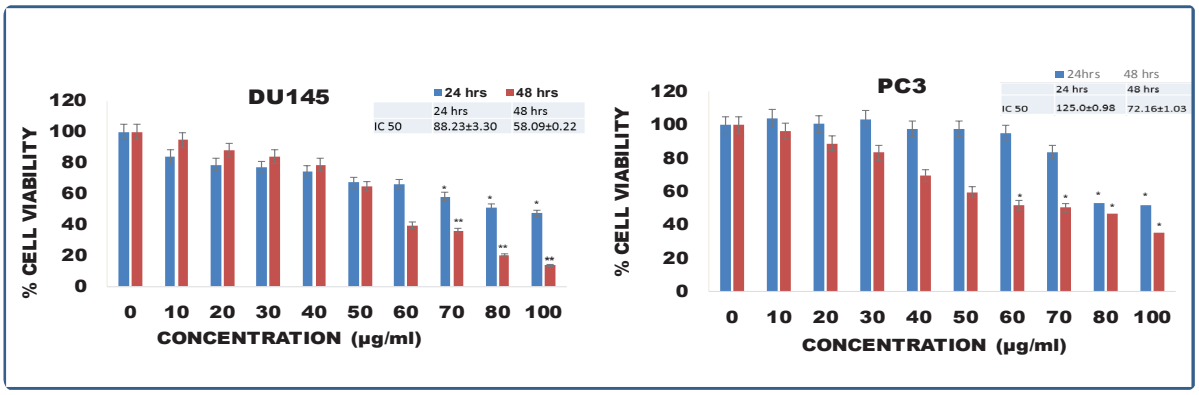

Figure 1: CVE inhibited the cell viability of prostate cancer cell DU145 and PC3 concentration-dependently at 24 and 48 hrs. time-points using MTT assay; Mean ± SEM; n=3, $p \leq 0.05$

V/ FITC assay, CVE (40 and 80 µg/ml) treated cells showed a concentration- dependent increase in the fraction of apoptotic DNA as depicted in Figure 3. This was corroborated by a concentration-dependent activation of Caspase 3 and PARP in both DU145 and C4-2 PCa cells at 48 hrs; and a concomitant activation of CDKI-p21 (Figure 4). Equal protein loading was confirmed by reprobing for GAPDH, a housekeeping protein.

CVE modulated the proteome of PCa Cells

LC/MS/MS analysis was performed to evaluate the effect of CVE on the proteome of PCa cells. This resulted in the identification of a total of 1012 proteins with a 0.05 confidence interval (CI). These proteins were then further screened based on fold change. A total of 16 proteins showing statistically significant ($p < 0.05$) change ≥ 1.7-fold (Table 1) were selected for further analysis. Details of these proteins including their protein ID, protein name, number of unique peptides and fold change upon CVE treatment are presented Table 1. The fold change increase or decrease in CVE- modulated proteins are represented in Figure 5a. The most significantly upregulated protein was the Transaldolase (TALDO) protein with a fold change of 2.4.

CVE modulates several metabolic networks including the Pentose Phosphate Pathway in PCa cells

Using the IPA software, canonical pathway analysis, putative networking and protein-protein relationships of the differentially expressed proteins were assessed. The 16 selected proteins (Table 1) was uploaded into the IPA module with their corresponding Swiss-

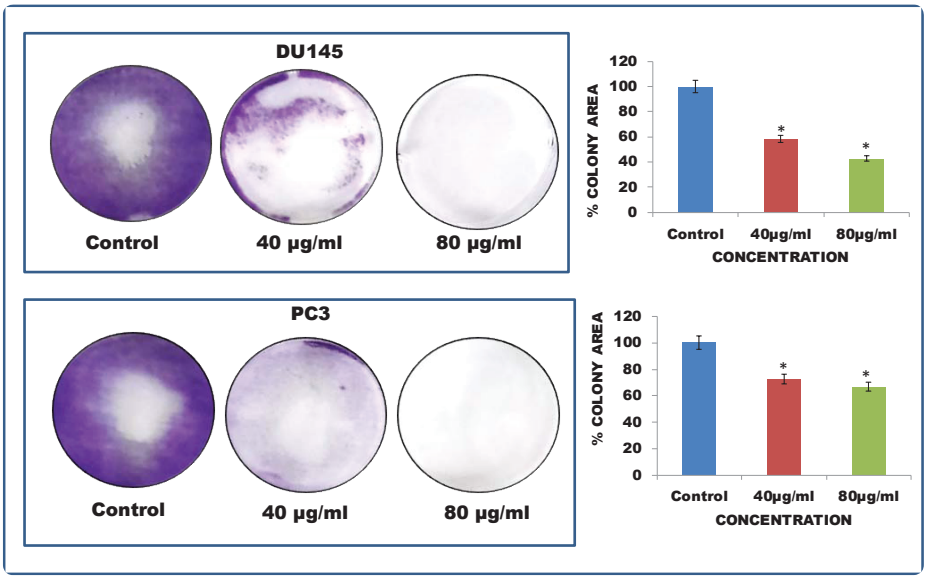

Figure 2: CVE inhibited formation of colonies in prostate cancer cell DU145 and PC3; Mean ± SEM; n=3, p ≤ 0.05

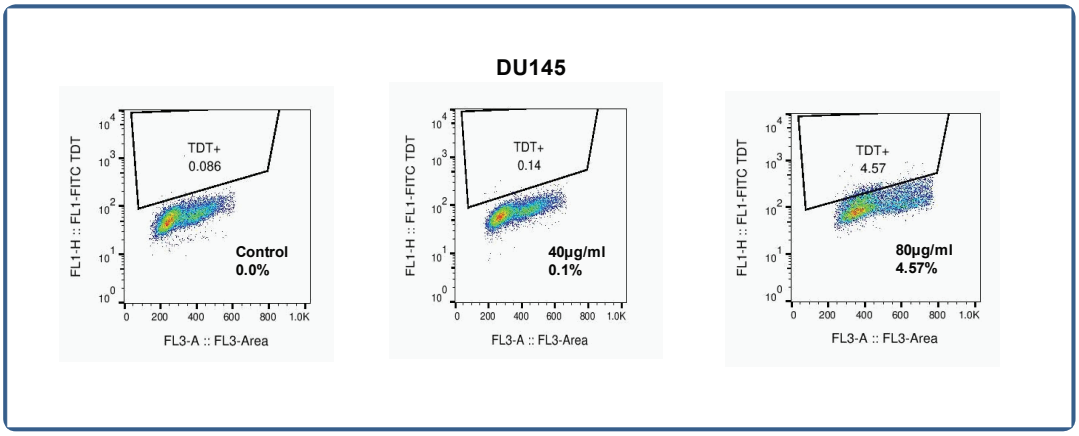

Figure 3: DU145 cells treated with CVE (40 & 80 µg/ml: 24h) labelled with FITC and analyzed by flow cytometry. Percentage of apoptotic cells shown.

Prot IDs and respective fold changes to map proteins into biological networks and identify key functional pathways. The EIF2 signaling, TCA cycle II and the Pentose phosphate pathway were among the 14 canonical pathways linked to CVE-modulated proteins (Figure 5B).

The 16 identified proteins annotated with the GO terms were explored for their biological functions using PANTHER classification system. A scheme showing the distribution of proteins among molecular functions, biological processes and protein classes are shown in Figure 6 respectively. Most of the differentially regulated proteins, when classified based on biological functions were found

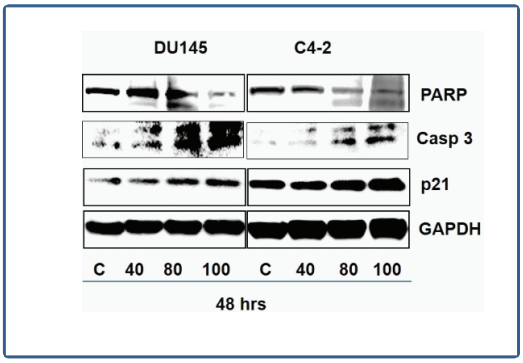

Figure 4: CVE induced a concentration-dependent cleavage of PARP and Caspase 3 in both DU145 and C4-2 cell; and a concomitant upregulation of CDKI p21 in both prostate cancer cells at 48 hrs.

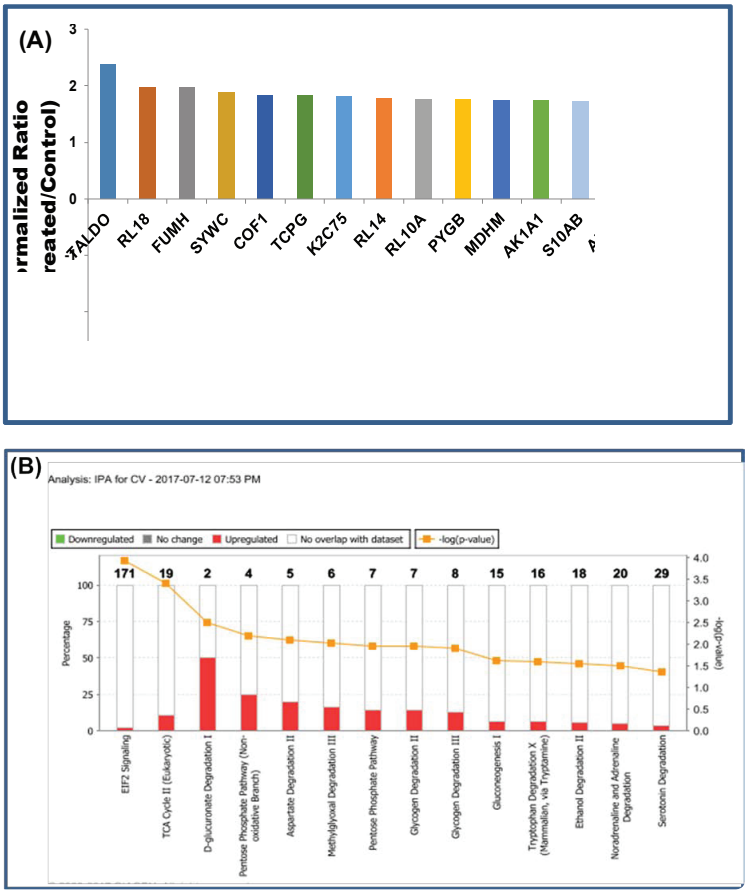

Figure 5: *CVE modulates the proteome of* PC3 *cells.* (A) Proteins showing > 1.7-fold change in abundance with CVE treatment (95% confidence interval and *p*-value. (B) IPA was used to classify the proteins on the basis of functional pathways affected.

to be involved in metabolic process (34%); cellular processes (33%) amongst others. Based on molecular functions, 38% were involved in structural molecule activity; 29% involved in binding and according to protein class, nucleic acid binding (25%) and cytoskeleton protein topped the chart.

Discussion

Despite a long history of use of medicinal plants, scientific research for their beneficial health effects has gained momentum only in the recent decades. Numerous studies have been undertaken, which range from isolation of active compounds, delineation of the mechanistic basis of their anticancer effect *in vitro* and *in vivo* and finally testing their efficacy in clinical trials. From these data, it is now evident that plants contain extractable biochemical and bioactive compounds, which can effectively target various human ailments including cancer. Thus there now is avid interest in therapeutic validation of plant-based compounds given the possibility of revealing new drug leads with better safety profiles [18]. Phytochemical screening of CVE showed the presence of saponins, flavonoids and phenols amongst others phytochemicals [19]. Saponins, the most abundant phytochemicals in CVE, flavonoids and phenols reportedly possess hypoglycemic, hypolipidemic, antioxidant and anticarcinogenic properties [20]. The present study was therefore designed to evaluate the cytotoxic activity of CVE and identify the molecular basis of its effect in PCa cells.

In *in vitro* studies, CVE treatment resulted in marked growth inhibition in PC3 and DU145 cell lines at extended time-points. Uncontrolled cell growth and high proliferative index, a common feature of cancer cells is linked intricately to loss of normal cell cycle control [21]. Modulation of cell cycle could therefore serve as an effective method for regulation of growth and proliferation. Cell cycle regulators, including the CDKs, a family of protein kinases controlling ordered cell cycle progression, have been investigated as mechanistic targets [22]. Our studies show that CVE treated cells show increased expression of CDK inhibitor p21, suggesting an effect of the extract on the cell cycle machinery. Further evidence of its growth inhibitory potential came from a visible lack of clonogenic capacity in CVE treated cells. Consistently, treatment of PCa cells with CVE resulted in induction of apoptosis as assessed by concentration-dependent cleavage of Caspase 3 and PARP, establishing the mechanism of cell death. The antiproliferative activity of dietary fatty acids from *Clerodendrum volubile* against human breast cancer [23] and glioblastoma cells have been reported [24]. However, what component of the extract is driving this effect in PCa cells remains to be ascertained.

Proteomic studies yielded 16 proteins that were modulated upon CVE treatment. Of these, the TALDO and Fumarate hydratase (FH) enzymes were the most significantly upregulated targets. The enzyme TALDO, component of the non-oxidative part of PPP, catalyzes the

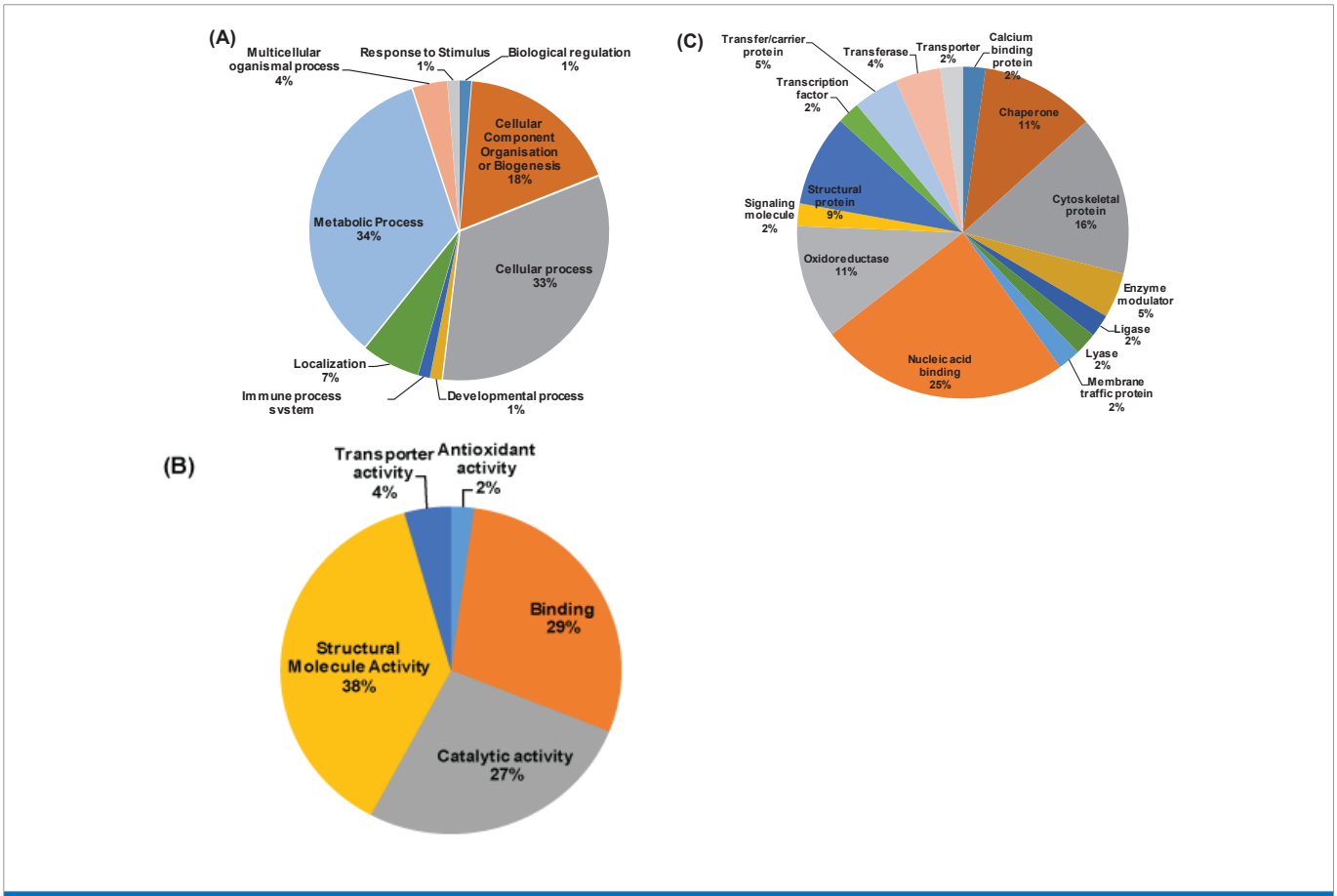

Figure 6: Gene Ontology analysis of proteome changes. Identified proteins showing ≥ 1.7-fold change were systematized based on: (A) Biological process (B) Molecular function (C) Protein classes by PANTHER classification system.

Table 1: Details of Proteins modulated upon *Clerodendrum volubile* (80µg/ml) treatment, showing ≥ 1.7 fold up- or down-regulation with statistical significance at *p*-value < 0.05.

S/No	Protein ID	Gene Name	Protein Description	Unique Peptides	Fold change
1	P37837	TALDO	Transaldolase	2	2.40
2	Q07020	RL18	60S ribosomal protein	3	1.99
3	P07954	FUMH	Fumarate hydratase_mitochondrial	2	1.97
4	P23381	SYWC	Tryptophan__tRNA ligase_ cytoplasmic	2	1.89
5	P23528	COF1	Cofilin_1 OS=Homo sapiens	3	1.82
6	P49368	TCPG	T_complex protein 1 subunit gamma	5	1.82
7	O95678	K2C75	Keratin_type II cytoskeletal	6	1.82
8	P50914	RL14	60S ribosomal protein	2	1.78
9	P62906	RL10A	60S ribosomal protein	3	1.76
10	P11216	PYGB	Glycogen Phosphorylase_ brain	9	1.76
11	P40926	MDHM	Malate dehydrogenase_ mitochondrial	3	1.75
12	P14550	AK1A1	Alcohol dehydrogenase	2	1.74
13	P31949	S10AB	Protein S100_A11	2	1.73
14	P02768	ALBU	Serum albumin	2	-1.73
15	P51991	ROA3	Heterogenous nuclear ribonucleoprotein A3	3	-1.79
16	P07910	HNRPC	Heterogenous nuclear ribonucleoproteins	2	-2.57

reversible transfer of a three-carbon unit 'dihydroxyacetone' between various sugar phosphates. An important function of TALDO is to sustain cellular homeostasis through generation of NADPH, which in turn maintains glutathione at a reduced state [25]. Studies in Jurkat T cells showed that reduced glutathione levels and sensitivity to apoptosis were regulated by changes in TALDO expression.

Overexpression of TALDO suppressed G6PD and 6-phosphogluconate dehydrogenase activities, decreased NADPH and GSH levels and rendered cell susceptible to apoptosis [26]. In a similar study, it was shown that TALDO regulated Fas-induced apoptosis by controlling the balance between mitochondrial reactive oxygen species production and metabolic supply of reducing equivalents generated through the pentose pathway [27]. This reciprocal relationship was also noted in xeroderma pigmentosum cells where high TALDO levels contributed to NADPH depletion and low catalase activity. Our results show that TALDO levels are significantly increased in CVE treated cells. It is plausible that CVE-mediated increase in TALDO results in reduced levels of NADPH and GSH levels, increased oxidative stress and activation of apoptotic pathways.

The enzyme FH catalyzes the conversion of fumarate to malate in the TCA cycle and is considered a potent tumor suppressor protein [28]. Germ line mutation of the gene encoding FH is linked to hereditary cancer syndrome known as hereditary leiomyomatosis and renal cell cancer. Biallelic FH inactivation in these tumors result in the accumulation of fumarate which then confers a proliferative signal to cells through disruption of ferritin signaling [29]. Studies in mouse and human cells have further demonstrated that loss of FH and accumulation of fumarate elicits an epithelial-to-mesenchymal-transition phenotype associated with cancer invasion, and metastasis [30]. Our data indicates that an increase in FH in CVE treated PCa cells is associated with growth inhibition. How altered levels of these enzyme affect PCa cell viability is presently unknown. Further studies directed at estimating the level of glucose utilization, its uptake and transport in CVE treated cells and the status of TCA cycle intermediates will help elucidate the precise mechanism through which the extract modulates biochemical signals and affects cell survival.

Taken together, our studies have provided for the first time preliminary evidence of the growth inhibitory potential of CVE and identifies signaling pathways altered in CVE-treated PCa cells.

Acknowledgement

The authors' wishes to thank Prof. Ibiyemi of the Department of Chemistry, University of Ilorin for availing us the use of his laboratory where the plant extract was prepared and Mr. Femi Adesua for his technical assistance during the plant preparation phase of the experiment.

Disclosure Statement

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

Author Contributions

Olufunke Olorundare is the Principal investigator of this study and reviewed the writing of the manuscript; Hasan Mukhtar is a co-investigator of this study and grant awarded for this study. Saheed Afolabi carried out the study in the laboratory of Hasan Mukhtar. Deeba Syed supervised the experimental framework and contributed greatly to the writing of the manuscript with Saheed Afolabi. Saheed Afolabi and Gideon Gyebi worked on the plant preparation phase of the research work.

Funding

We are grateful to Tertiary Education Trust Fund (TETFUND)

Nigeria, for the grant (TETFUND//NRF/UIL/ILORIN/STI/VOL.1/B2.20.12) awarded for this work.

References

1. Stewart, BW., Wild, CP. (2014) World cancer report. Retrieved from https://publications.iarc.fr/Non-Series-Publications/World-Cancer-Reports/World-Cancer-Report-2014
2. Siegel, RL., Miller, KD., Jemal, A. (2016) Cancer statistics. CA Cancer J Clin, 66(1): 7-30.
3. Kushi, LH., Doyle, C., McCullough, M., Rock, CL., Demark-Wahnefried, W., Bandera, EV., et al. (2012) American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. CA Cancer J Clin, 62(1): 30-67.
4. Nevedomskaya, E., Baumgart, S., Haendler, B. (2018) Recent advances in prostate cancer treatment and drug discovery. Int J Mol Sci, 19(5): 1359.
5. Taitt, HE. (2018) Global trends and prostate cancer: A review of incidence, detection, and mortality as influenced by race, ethnicity, and geographic location. Am J Mens Health, 12(6): 1807-1823.
6. Oreagba, IA., Oshikoya, KA., Amachree, M. (2011) Herbal medicine use among urban residents in Lagos, Nigeria. BMC Complement Altern Med, 11(1): 117.
7. Osemene, KP., Elujoba, AA., Ilori, MO. (2013) A comparative assessment of herbal and orthodox medicines in Nigeria. Niger J Nat Prod Med, 17(1): 77-81.
8. Duru, CB., Diwe, KC., Uwakwe, KA., Duru, CA., Merenu, IA., Iwu, AC., et al. (2016) Combined orthodox and traditional medicine use among households in Orlu, Imo State, Nigeria: prevalence and determinants. World J Prev Med, 4(1): 5-11.
9. Payyappallimana, U. (2010) Role of traditional medicine in primary health care: an overview of perspectives and challenging. AYISS, 14: 6.
10. Kamal, Z., Bairage, JJ., Moniruzzaman, DP., Islam, M., Faruque, M., Islam, MR., et al. (2014) Ethnomedicinal practices of a folk medicinal practitioner in Pabna district, Bangladesh. World J Pharm Pharmaceut Sci, 3(12): 73-85.
11. Mohsenzadegan, M., Seif, F., Farajollahi, MM., Khoshmirsafa, M. (2018) Anti-oxidants as chemopreventive agents in prostate cancer: A gap between preclinical and clinical studies. Recent Pat Anti Canc, 13(2): 224-239.
12. Yuan, H., Ma, Q., Ye, L., Piao, G. (2016) The traditional medicine and modern medicine from natural products. Molecules, 21(5): 559.
13. Atanasov, AG., Waltenberger, B., Pferschy-Wenzig, EM., Linder, T., Wawrosch, C., Uhrin, P., et al. (2015) Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv, 33(8): 1582-1614.
14. Erukainure, LO., Oke, OV., Owolabi, FO., Adenekan, SO. (2010) Antioxidant nutrient properties and antioxidant activities of obenetete (Clerodendrum volubile), a nonconventional leafy vegetable consumed in Nigeria. Afr J Food Agric Nutr Dev, 10(10).
15. Burkill, HM. (1994) The useful plants of west tropical Africa. Volume 2: Families EI. Royal Botanic Gardens.
16. Shabbir, M., Syed, DN., Lall, RK., Khan, MR., Mukhtar, H. (2015) Potent anti-proliferative, pro-apoptotic activity of the Maytenus royleanus extract against prostate cancer cells: evidence in in-vitro and in-vivo models. PloS one, 10(3): e0119859.
17. Singh, CK., Kaur, S., George, J., Nihal, M., Hahn, MC., Scarlett, CO., et al. (2017) Molecular signatures of sanguinarine in human pancreatic cancer cells: a large scale label-free comparative proteomics approach. Oncotarget, 6(12): 10335.
18. Dhama, K., Latheef, SK., Mani, S., Samad, HA., Karthik, K., Tiwari, R., et al. (2015). Multiple beneficial applications and modes of action of herbs in poultry health and production-A review. Int J Pharmacol, 11(3): 152-176.
19. Ogunwa, TH., Adeyelu, TT., Fasimoye, RY., Oyewale, MB., Ademoye, TA., Ilesanmi, OC., et al. (2016) Phytochemical evaluation and in vitro antioxidant status of Clerodendrum volubile (an indigenous medicinal plant). Pak J Pharm Res, 2: 77-88.
20. Elekofehinti, OO., Adanlawo, IG., Fakoya, A., Saliu, JA., Sodehinde, SA. (2012) Effects of Saponins from Solanum anguivi Lam fruit on heart and kidney superoxide dismutase, catalase and malondialdehyde in rat. Res J Pharm Biol Chem Sci, 4(4): 530-533.
21. Hanahan, D., Weinberg, RA (2011) Hallmarks of cancer: the next generation. Cell, 144(5): 646-674.
22. Asadi-Samani, M., Rafieian-Kopaei, M., Lorigooini, Z., Shirzad, H (2018) A screening of growth inhibitory activity of Iranian medicinal plants on prostate cancer cell lines. BioMedicine, 8(2): 8.
23. Erukainure, OL., Zaruwa, MZ., Choudhary, MI., Naqvi, SA., Ashraf, N., Hafizur, RM., et al. (2016) Dietary fatty acids from leaves of Clerodendrum volubile induce cell cycle arrest, downregulate matrix metalloproteinase-9 expression, and modulate redox status in human breast cancer. Nutr Cancer, 68(4): 634- 645.
24. Erukainure, OL., Ashraf, N., Naqvi, AS., Zaruwa, MZ., Muhammad, A., Odusote, AD., et al. (2018) Fatty Acids Rich Extract From Clerodendrum volubile Suppresses Cell Migration; Abates Oxidative Stress; and Regulates Cell Cycle Progression in Glioblastoma Multiforme (U87 MG) Cells. Front Pharmacol, 9: 251.
25. Rafehi, H., Orlowski, C., Georgiadis, GT., Ververis, K., El-Osta, A., Karagiannis, TC. (2011) Clonogenic assay: adherent cells. J Vis Exp, 13(49): e2573.
26. Banki, K., Hutter, E., Colombo, E., Gonchoroff, NJ., Perl, A. (1996) Glutathione levels and sensitivity to apoptosis are regulated by changes in transaldolase expression. J Biol Chem, 271(51): 32994-33001.
27. Banki, K., Hutter, E., Gonchoroff, NJ., Perl, A. (1999) Elevation of mitochondrial transmembrane potential and reactive oxygen intermediate levels are early events and occur independently from activation of caspases in Fas signaling. J Immunol, 162(3): 1466-1479.
28. Schmidt, C., Sciacovelli, M., Frezza, C. (2019) Fumarate hydratase in cancer: A multifaceted tumor suppressor. In Semin Cell Dev Biol. Academic Press.
29. Kerins, MJ., Vashisht, AA., Liang, BX., Duckworth, SJ., Praslicka, BJ., Wohlschlegel, JA., et al. (2017) Fumarate mediates a chronic proliferative signal in fumarate hydratase- inactivated cancer cells by increasing transcription and translation of ferritin genes. Molecular and cellular biology, 37 (11): e00079-17.
30. Sciacovelli, M., Gonçalves, E., Johnson, TI., Zecchini, VR., da Costa, AS., Gaude, E., et al. (2016) Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition. Nature, 537(7621): 544-547.