RESEARCH ARTICLE

Unveiling Cytotoxic Potential of Herbal Extracts: *Plectronia parviflora* and *Agave cantula* against MCF and HCT Cell Lines

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ABSTRACT

Cancer is a global health challenge that requires novel therapeutic approaches. This study investigates hydroalcoholic extracts and compounds isolated from *Plectronia parviflora* and *Agave cantula*. Using them as cancer therapies will shed light on their potential as cytotoxic agents. Microscopic confirmation of complete dissociation followed DMEM medium supplemented with 10% FBS cultivation of HCT-15 and MCF-7 cell lines. As controls, cells were introduced without samples at varying concentrations. After the medium was removed, a 24-hour incubation was followed by rinsing the cells in PBS. Trypan blue stain (0.4%) was used to determine cell viability. Upon introducing cells to fresh medium, triplicate samples were introduced. After 18 hours of incubation at 37 ± 1°C, MTT (mg/mL) was added to all wells and incubated for an additional 4 hours. Subsequently, DMSO was introduced, and absorbance readings were taken at 570 nm using a photometer. Cytotoxicity and cell viability were calculated using established formulas. Cytotoxicity analysis revealed distinct patterns in both HCT-15 and MCF-7 cell lines. Among the extracts, hydroalcoholic extracts of *P. parviflora* exhibited notable cytotoxicity, with IC_{50} values of 108.46 and 133.37 µg/mL for HCT-15 and MCF-7, respectively. *A. cantula* extracts displayed variable cytotoxicity, with IC_{50} values 115.35 and 115.94 µg/mL for HCT-15 and MCF-7. Notably, isolated compounds from these plants exhibited unique cytotoxic profiles with IC_{50} values of IF-PL-57.82 and 53.72 mg/mL and IF-AC-72.81 and 72.36 mg/mL for HCT-15 and MCF-7. As potential cancer therapy agents, hydroalcoholic extracts of *P. parviflora* and *A. cantula* reveal cytotoxic potential. The distinctive cytotoxicity profiles among extracts and isolated compounds underscore the complexity of their mechanisms. Further exploration of the mechanisms and synergies with existing anticancer drugs is required to elucidate these findings, offering exciting prospects for the future.

Keywords: HCT-15, MCF-7, Cytotoxicity, *Plectronia, Agave*.

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INTRODUCTION

Cancer is the second leading cause of death worldwide, behind heart disease. There has been an increase in the prevalence of cancer and the number of people affected by it. Cancer affected 1.6 million Americans in 2014 alone, and 0.5 million of them still suffer from this devastating disease today. There are, therefore many health challenges that face societies all over the world when it comes to fighting cancer. There are a number of reasons why cancer is a complex disease, especially due to its heterogeneous nature at the tissue level, which can pose a formidable challenge in terms of accurate diagnosis and effective treatment.[1] There are an estimated 9% of cancer-related deaths in the year 2020 that can be attributed to colorectal cancer (CRC). Increasing numbers of CRC cases are emerging among the elderly population, which is alarming as the numbers of cases of CRC are steadily on the rise. Various studies have projected that by the year 2035, there will be a more than two-fold increase in the global incidence of CRC, with the majority of these increases coming from countries that are underdeveloped.[2,3]

It is estimated that colorectal cancer and lung cancer comprise 11.6% of all diagnoses of cancers in both genders and are, therefore the two most commonly diagnosed cancers. On the other hand, prostate cancer takes second place for men (7.1%), while breast cancer takes the lead for women (11.6%), which is followed closely by breast cancer (11.6%). Among the three most common types of cancer in terms of diagnosis, CRC is the third most prevalent, accounting for 6.1% of all cases. There is no doubt that CRC is the second most common cancer-related cause of death, accounting for 9.2% of those who lose their lives to cancer. Several studies are indicating that the number of deaths caused by rectal and colon cancers

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is expected to surge by 71.5 and 60%, respectively, by the year 2035. This might be a cause for concern as there is a worrying trend.[4] There are a lot of cases of breast cancer in oncology, with approximately 36% of all oncology patients having breast cancer. Breast cancer accounts for a significant proportion of all cancer patients. According to estimates, 2.189 million women worldwide are estimated to have received breast cancer diagnoses in 2018 alone.[5] Notably, the 5-year survival rate for breast cancer in Poland stands at 78.5%, a figure that significantly deviates from survival rates observed in other developed nations.[6] The incidence of breast cancer-related deaths, showing an approximately 88% higher incidence rate, is more pronounced in transitioning countries. Furthermore, it ranks as the fifth leading cause of cancer-related deaths worldwide, with an estimated 2.3 million new cases surfacing globally.[7] The global rise in CRC cases is a concerning trend influenced by a combination of environmental and genetic factors. Notably, the risk of developing CRC escalates with age, particularly in individuals with long-standing ulcerative colitis and Crohn's disease.[8]

Presently, the diagnostic landscape can only identify approximately 40% of CRC cases in their early stages, and even after surgery and post-surgery treatment, the possibility of CRC recurrence looms.[9] While chemotherapeutic medications are designed to target cancer cells, they often exact collateral damage on healthy cells within the vicinity. Moreover, modern chemotherapies have encountered resistance in nearly all CRC patients, undermining the effectiveness of anticancer drugs and ultimately resulting in chemotherapy failure.[10] Chemotherapy-induced side effects are common among cancer patients and can range from troublesome to life-threatening, often manifesting when patients are in their home environments.[11] Several side effects are associated with this medication, including nausea, vomiting, fatigue, diminished appetite, changes in taste perception, baldness, and dry mouth. Achieving effective management of these side effects can improve tumor therapy's efficacy.[12]

Traditional medicine often relies on plant-based products due to their ready availability in rural areas and cost-effectiveness compared to modern therapeutic drugs.[13] One such plant, *Plectronia parviflora* (Lam.) Bedd., also known synonymously as *Canthium coromandelicum* (Burm.f.) Alston, belonging to the Rubiaceae family[14], has garnered attention for its diverse medicinal properties. *C. coromandelicum* leaves have been recognized for their antibacterial and antiretroviral activities, antiobesity effects, anti-diabetic properties, wound healing capabilities, and antioxidant activities.[14] Notable chemical constituents within this plant include kaempferol, p β-sitosterol, hytol and hexadecanoic acid.[15] Traditionally, this plant has been employed in India to treat a spectrum of ailments, including gut worms in kids, as well as serving as a diuretic agent, remedy for skin infections, headache, fever, snake bites, indigestion, nausea, dysuria, impotence, and gastrointestinal disorders like gastric ulcer and constipation.[16] *Agave cantula*, a member of the Agavaceae family, is a scapigerous herb with a perennial nature, commonly found in wastelands and along roadsides. It is also frequently cultivated as a fencing plant.[17] It is believed that various parts of this plant have been used traditionally as medicines for various medical issues, with different parts of the plant being used for various purposes. It is believed that folklore claims that this mixture has a range of traditional uses, including purgative, anti-scurvy, anti-syphilis, anti-edema, anti-retention of urine, and anticancer.[18] Several components have been identified in *A. cantula* when chemically analyzed. These components include flavonoid-type glycosides, sterol-type glycosides, hecogenin, and tigogenin, among others.[19] In addition, the plant's aqueous and alcoholic extracts have been examined by researchers for their potential to exhibit cytotoxic properties. According to the brine shrimp method, we were able to determine the LC_{50} values for the aqueous extract and the alcoholic extract of the extract using a 15 mg LC_{50} value and 12.5 mg LC_{50} value, respectively.[20] *A. cantula* has been found to have potential cytotoxic effects, which highlights its potential importance in medicinal research and in traditional usage. These findings highlight the potential cytotoxic effects of *A. cantula*. The purpose of this study has been to determine the cytotoxicity of hydroalcoholic extracts from *Plectronia* and *Agave*, as well as isolated compounds from the plants, against HCT-15 and MCF-7 cells, as well as their interaction with each other.

MATERIAL AND METHODS

Extraction and Isolation

Fresh leaves of *Plectronia* and *Agave* were collected, authenticated and dried in shade for 5 days. About 50 gm of the powdered dried leaves were extracted using pet-ether, methanol and combination of water and alcohol (1:1) to prepare pet ether extract of *Plectronia* and *Agave* (PEPL-13.43% w/w and PEAC-12.09% w/w), Methanol extract of *Plectronia* and *Agave* (MEPL-23.32% and MEAC-24.57% w/w) and hydroalcoholic extracts (HEPL-24.82% and HEAC-24.99% w/w), respectively. Upon testing for cytotoxicity HEPL and HEAC were subjected to isolation of compounds using column chromatography using dichloromethane: Acetone: Water (6:1:3). Fractions from HEPL 1-4 (1.25 gm), 5 to 9 (4.33 gm) and 10 to 12 (2.12 gm) were pooled on the basis of color similarity and dried using rotary evaporator and compound was isolated from a second fraction (IF-PL). Fractions from HEAC 1 to 3 (1.12 gm), 4 to 9 (1.27 gm), 10 to 13 (4.48 gm) and 14 to 16 (0.96 gm) were pooled on the basis of color similarity and dried using a rotary evaporator. The compound was isolated from third fraction (IF-AC) and stored in an air-tight container at 4°C for further use.

Cell lines

HCT-15 and MCF-7 were procured from NCCS, Pune and were maintained in DMEM medium enriched with FBS and 1-mL of each antibiotic solution. Microscopic examination was conducted to ensure the absence of bacterial and fungal contamination in the cell cultures. The cell layers underwent a thorough washing step with phosphate buffer saline (PBS), utilizing a volume equal to half of that of the culture medium.

Subsequently, EDTA was introduced into the culture cells, and the flask underwent circular rotation to ensure even distribution of the added substance. This mixture was then incubated for an additional 5 to 10 minutes within the incubator. The cells were subsequently resuspended in a small volume of fresh serum. A quantity of 50 to 100 µL of the prepared mixture was extracted for cell counting. The desired number of cells was then isolated from the flask to facilitate the ensuing study.

In-vitro MTT Assay for Cytotoxicity Activity

The in vitro cytotoxicity assay for the provided test sample was conducted in accordance with the guidelines specified in ISO 10993:5. The protocol included the replacement of the culture medium in the cells with fresh medium. Triplicates of the test sample were then applied to the cells. Following an incubation period at 37 ± 1°C for 18 hours, MTT (mg/mL) was added to all the wells and allowed to incubate for an additional 4 hours. Subsequently, DMSO was introduced into the wells, and the absorbance was measured at 570 nm using a photometer.[21] The cytotoxicity and cell viability calculation was carried out using the formula provided.

Cytotoxicity = [(Control-Treated)/ Control] *100 Cell viability = (Treated/ Control)*100

Trypan Blue Assay

Cells were cultured in DMEM medium supplemented with 10% FBS. A mixture of trypsin (0.25%) and versene (0.1%) in equal volumes was used to detach the cells, and their dissociation was confirmed under a microscope. Approximately, 105 cells were seeded per well and incubated at 37°C for 24 hours. Samples were added at different concentrations in duplicate, with cells without samples serving as a control. The plate was then incubated for an additional 24 hours. After incubation, the medium was completely removed, and cells were rinsed with PBS. Trypan blue stain (0.4%) was added to each well, and observations were made under an inverted phase-contrast microscope.[22]

Statistical Analysis

The values were subjected to statistical analysis to ensure variance and expressed as Mean ± SEM.

RESULTS AND DISCUSSION

In this study, we examined the cytotoxic effects of extracts from *P. parviflora* (PEPL, MEPL, HEPL) and *A. cantula* (PEAC, MEAC, HEAC), the standard drug 5-fluorouracil (5-FU), and isolated compounds from *Plectronia* (IF-PL) and *Agave* (IF-AC) on HCT-15 cell lines and MCF-7 cell lines.

Effect of Extracts on HCT-15 Cell lines

Our results of HCT-15 cell lines revealed varying degrees of cytotoxicity among these compounds (Table 1). Among the extracts, HEPL and HEAC demonstrated the highest cytotoxicity, resulting in a remarkable reduction in cell viability. Interestingly, this extract outperformed the standard 5-FU in terms of cytotoxicity. Notably, IF-PL and IF-AC isolated from HEPL and HEAC, respectively showed potent cytotoxicity compared to the extracts and 5-FU. These findings suggest that HEPL may possess potent cytotoxic properties against HCT-15 cell lines (Figure 1), potentially surpassing the efficacy of the standard drug 5-FU, while the isolated compounds, IF-PL and IF-AC, exhibited intriguing cytotoxic activity.

Effect of Extracts on MCF-7 Cell lines

In our investigation of cytotoxicity on MCF-7 cell lines, certain extracts stood out for their higher cytotoxicity, reflected in lower %cell viability values. Notably, HEPL

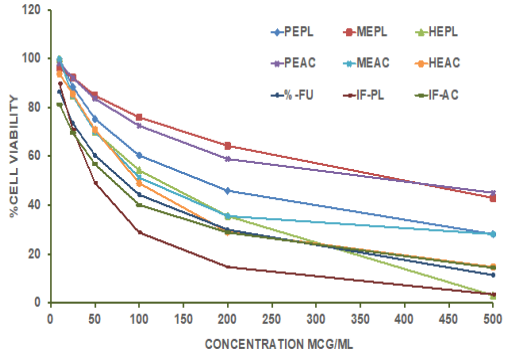


Figure 1: Effect of extracts and isolated compound of *Plectronia and Agave* on the HCT-15 cell lines

Table 1: *In-vitro* cytotoxicity of *Plectronia and Agave* on the HCT-15 cell lines

Sample	*Concentration (µg/mL)*					
	500	*200*	*100*	*50*	20	10
PEPL	99.84 ± 8.38	88.42 ± 7.42	75.33 ± 6.32	60.48 ± 5.08	45.92 ± 3.85	28.22 ± 2.37
MEPL	96.03 ± 8.06	92.28 ± 7.75	84.83 ± 7.12	76.09 ± 6.39	64.38 ± 5.4	43.04 ± 3.61
HEPL	99.65 ± 8.37	84.76 ± 7.11	70.02 ± 5.88	54.31 ± 4.56	35.55 ± 2.98	3.02 ± 0.25
PEAC	98.05 ± 8.23	92.05 ± 7.73	83.49 ± 7.01	72.53 ± 6.09	58.95 ± 4.95	45.06 ± 3.78
MEAC	99.81 ± 8.38	84.92 ± 7.13	70.16 ± 5.89	51.41 ± 4.31	35.63 ± 2.99	28.26 ± 2.37
HEAC	93.72 ± 7.87	85.52 ± 7.18	70.94 ± 5.95	49.05 ± 4.12	28.96 ± 2.43	14.66 ± 1.23
5-FU	86.533 ± 7.26	73.507 ± 6.17	60.431 ± 5.07	44.272 ± 3.71	30.09 ± 2.52	11.471 ± 0.96
IF-PL Isolated fraction-1	89.832 ± 7.54	70.947 ± 5.95	49.3 ± 4.14	28.928 ± 2.42	14.669 ± 1.23	3.493 ± 0.29
IF-AC Isolated fraction-2	81.21 ± 6.82	69.56 ± 5.84	56.86 ± 4.77	40.22 ± 3.37	28.87 ± 2.42	14.44 ± 1.21

showed results similar to previous cell lines, demonstrating the most pronounced cytotoxicity. Similarly, HEAC exhibited substantial cytotoxic effects, displaying %cell viability values comparable to the standard drug 5-FU. When comparing the extracts with the standard drug 5-FU, it's notable that extracts showed comparable or even higher cytotoxicity at specific concentrations. However, the isolated compounds, IF-PL and IF-AC, generally followed patterns similar to their respective parent extracts, emphasizing the complexity of their cytotoxic interactions with MCF-7 cells (Table 2 and Figure 2).

In vitro Cytotoxicity of Extracts and Compounds on Cell lines

The comparison of IC_{50} values across HCT-15 and MCF-7 cell lines reveals intriguing insights into the cytotoxic potential of the tested samples (Table 3). In the context of HCT-15 cells, HEPL exhibited the most pronounced cytotoxicity with the lowest IC_{50} value of 108.46 µg/mL, signifying its potency in inhibiting the growth of these cells. Furthermore, IF-PL (Isolated compound-1) from *Plectronia* also demonstrated substantial cytotoxicity, displaying a relatively low IC_{50} value of 57.82 µg/mL, suggesting its potential as an effective cytotoxic agent against HCT-15 cells. Notably, the standard drug 5-FU showed strong cytotoxicity with an IC_{50} value of 83.05 µg/mL, although slightly higher than that of HEPL and IF-PL. Turning our attention to MCF-7 cells, HEPL again exhibited significant cytotoxicity, as reflected in its IC_{50} value of 133.37 µg/mL. IF-PL retained its potency on this cell line, with an IC50 value of 53.72 µg/mL. In comparison, the standard drug 5-FU displayed an IC_{50} value of 85.19 µg/mL, indicating robust cytotoxicity. These findings highlight the diverse cytotoxic profiles of the tested samples on different cell lines, emphasizing the importance of tailored approaches in potential cancer therapeutics.

Previous research has provided valuable insights into the potential therapeutic benefits of hydroalcoholic extracts from *Plectronia* and *Agave* leaves, particularly in the context of carcinoma treatment. These studies have uncovered noteworthy anti – inflammatory and apoptotic activities associated with extract of *Plectronia*. It has been demonstrated that the extract efficiently induces apoptosis by modulating oxidative damage and effectively suppressing inflammation, presenting promising prospects for hepatocellular carcinoma therapy *in-vitro*.[14] Moreover, in vitro investigations have shed light on the cytotoxic properties of *Plectronia*, particularly on SHSY5Y cell lines. These studies have revealed an IC_{50} value of less than 50 µg/mL, underscoring its potential as

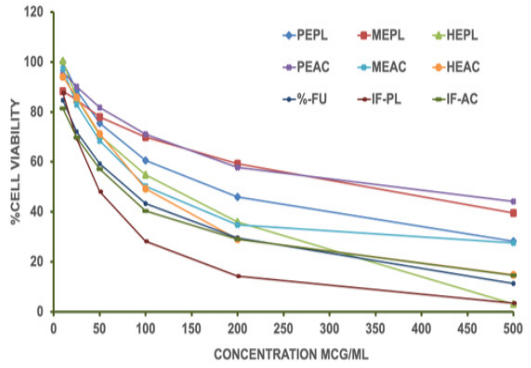


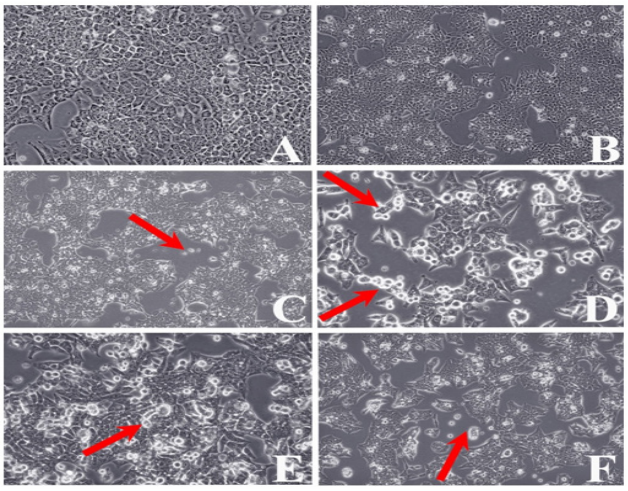
Figure 2: Effect of extracts and isolated compound of *Plectronia* and *Agave* on the MCF-7 cell lines

Table 3: Cytotoxicity of *Plectronia* and *Agave* on HCT and MCF cell lines

Sample	*IC50 (µg/ml)*	
	HCT-15	*MCF-7*
PEPL	198.78	173.22
MEPL	406.2	351.79
HEPL	108.46	133.37
PEAC	406.03	390.06
MEAC	127.55	121.94
HEAC	115.35	115.94
5-FU	83.05	85.19
IF-PL Isolated fraction-1	57.82	53.72
IF-AC Isolated fraction-2	72.81	72.36

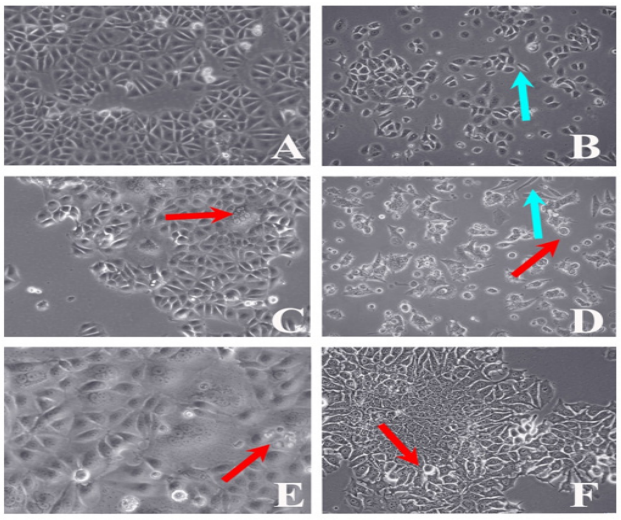
Table 2: *In-vitro* cytotoxicity of *Plectronia* and *Agave* on the MCF-7 cell lines

Sample	*Concentration (µg/mL)*					
	500	*200*	*100*	*50*	*20*	*10*
PEPL	99.03 ± 8.82	88.34 ± 8.48	99.54 ± 8.80	96.08 ± 8.66	97.44 ± 8.82	94.01 ± 8.28
MEPL	88.59 ± 7.81	84.89 ± 8.15	85.52 ± 7.49	90.2 ± 8.13	82.88 ± 7.50	85.79 ± 7.55
HEPL	75.48 ± 6.65	78.04 ± 7.49	70.65 ± 6.18	81.82 ± 7.37	68.47 ± 6.19	71.16 ± 6.26
PEAC	60.6 ± 5.34	70 ± 6.72	54.79 ± 4.79	71.07 ± 6.40	50.17 ± 4.54	49.2 ± 4.33
MEAC	46.01 ± 4.05	59.22 ± 5.68	35.86 ± 3.14	57.77 ± 5.20	34.77 ± 3.14	29.05 ± 2.55
HEAC	28.27 ± 2.49	39.59 ± 3.80	3.047 ± 0.26	44.15 ± 3.98	27.58 ± 2.49	14.7 ± 1.29
5-FU	99.03 ± 8.82	88.34 ± 8.48	99.54 ± 8.80	96.08 ± 8.66	97.44 ± 8.82	94.01 ± 8.28
IF-PL Isolated fraction-1	88.59 ± 7.81	84.89 ± 8.15	85.52 ± 7.49	90.2 ± 8.13	82.88 ± 7.50	85.79 ± 7.55
IF-AC Isolated fraction-2	75.48 ± 6.65	78.04 ± 7.49	70.65 ± 6.18	81.82 ± 7.37	68.47 ± 6.19	71.16 ± 6.26



Red arrows indicate clustering and condensation of nucleus and cell membranes
(A. Normal), (B. HEPL), (C. HEAC), (D. 5-FU), 9E. IF-PL), (F. IF-AC)

Figure 3: Morphological changes of HCT-15 cell lines



(A. Normal), (B. HEPL), (C. HEAC), (D. 5-FU), (E. IF-PL), (F. IF-AC)

Figure 4: Morphological changes of MCF-7 cell lines

a cytotoxic agent.[23] Furthermore, the isolation of squalene from the leaves of this plant has shown significant promise. Squalene exhibited noteworthy potency against HepG2 cell lines, with an IC_{50} of 100 µg/mL, suggesting its potential utility in cancer research and treatment.[24-27] These findings collectively highlight the multifaceted therapeutic potential of *Plectronia* and its constituents in the field of oncology. On the other hand, *Agave* plant aqueous and alcoholic extracts were also investigated and proved for its anticancer activity on invitro using the brine shrimp method, the LC_{50} values were determined to be 15 mg for the aqueous extract and 12.5 mg for the alcoholic extract.[20] Thus further investigation needs to be concentrated on the identification of isolated compound from the plant.

Histopathological Studies

In our experiments, we also aimed to understand using the trypan blue assay, how the cellular morphology of healthy HCT-15 and MCF-7 cell lines was affected by exposure to the isolated fractions of both *P. parviflora* and *A. cantula*, as well as the standard drug 5-FU. Healthy cell lines typically display regular and intact cellular structures, including well-defined cell membranes and uniform cytoplasmic content. However, upon exposure to the isolated fractions from *Plectronia* (IF-PL) and *Agave* (IF-AC), we observed significant alterations in cellular morphology. Cells treated with these fractions exhibited various changes, including cell shrinkage, cytoplasmic condensation (marked with red arrows), and irregular cell membrane integrity and deformation (marked with blue arrows). This can include the formation of membrane blebs or irregularities in the cell surface. These alterations indicate that IF-PL and IF-AC induced notable structural changes in the cells, suggesting potential cytotoxic effects.

In the above Figure 3, 4, the Red arrows indicate clustering and condensation of nucleus; Blue arrows indicate morphological changes of cell structure including cell membrane.

CONCLUSION

In conclusion, the current study has delved into the remarkable potential of plant extracts, specifically hydroalcoholic extracts from *Plectrinia* and *Agave* leaves, in the area of cancer research and therapy. The findings underscore the multifaceted nature of these extracts, revealing their capacity to induce apoptosis, modulate oxidative stress, and mitigate inflammation. Moreover, the cytotoxic properties demonstrated on various cell lines, along with the promising results from squalene isolation, add weight to the notion that *Plectronia* and *Agave* holds significant promise as a source of cytotoxic and anticancer agents. Looking ahead, the scope for new work in this domain is vast. Future research endeavors could delve deeper into the molecular mechanisms governing the cytotoxic and apoptotic effects of *Plectronia* and *Agave* extracts, paving the way for the development of targeted therapies. Additionally, exploring the potential synergy of these extracts with existing anticancer drugs could offer innovative treatment strategies. Moreover, the investigation of impact on various cancer types and the exploration of additional bioactive compounds within the plant hold promise for expanding our understanding and harnessing the full potential of this natural resource in the fight against cancer. Ultimately, this study not only advances our knowledge but also underscores the rich prospects that lie ahead in the field of natural product-based cancer research.

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