FACTA UNIVERSITATIS
Series **Medicine and Biology** Vol. 26, No 1, 2024, pp. 1–7

UDC 547.66:615.451.1]:616-006.6
https://doi.org/10.22190/FUMB240126002D

Original Scientific Article

FERMENTATION ENHANCES THE ANTIPROLIFERATIVE ACTIVITIES OF *ANNONA SQUAMOSA* SEED POLYSACCHARIDES ON HUMAN MCF-7 AND RHABDOMYOSARCOMA

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Abstract. Cancer is a primary cause of death globally and a significant public health concern. Polysaccharides are complex carbohydrates found abundantly in natural sources, including plants, and have attracted interest due to their possible health advantages, including anticancer activities. *Annona squamosa*, also known as the sugar apple, has long been prized for its healing properties, thus an intriguing candidate for investigating its potential effects on cancer cells. Through boosting bioavailability, producing active metabolites, enriching the nutrient profile, altering the composition of bioactive substances, and influencing the gut microbiota, fermentation plays a crucial role in promoting the antiproliferative effects of food. Polysaccharide was extracted from the fermented and unfermented cotyledon and coat of *A. squamosa* seed, characterised by HPLC, and antiproliferative activity was investigated using the MTT assay on human breast adenocarcinoma (MCF-7) and rhabdomyosarcoma (RD) cell lines. The Vero cell line obtained from the kidney of a green monkey of African descent was used for selectivity. The polysaccharides displayed antiproliferative activity against the cancerous cell lines MCF-7 (breast cancer) and RD (rhabdomyosarcoma-soft tissue sarcoma), with IC_{50} values ranging from 27.10 ± 0.61 to 57.01 ± 0.06 µg/ml and a good selectivity for the cancer cells over normal body cells (Vero), with fermented better than unfermented. In conclusion, *A. squamosa* seed polysaccharides exhibited antiproliferative properties on MCF-7 and RD, which could be explored in developing a novel drug in cancer prevention and treatment.

Key words: *Annona squamosa*, cancer cell lines, fermentation, polysaccharides, antiproliferative activity

Introduction

A primary cause of death globally and a significant public health concern is cancer [1]. There is still a need for medicines that are more efficient and less harmful despite substantial advancements in cancer treatment. Natural materials, such as polysaccharides from various sources, have demonstrated encouraging anticancer activity and may be used to create novel cancer treatments [2]. Natural substances called polysaccharides, present in a wide range of plants and fungi, are attracting more and more interest due to their alleged anticancer abilities [3, 4]. The seeds of the tropical fruit tree known as *Annona squamosa* sometimes referred to as sugar apple, have long been valued for their therapeutic benefits and are a promising source of polysaccharides [5]. According to studies, Annona squamosa extracts from several plant sections , such as the leaves, seeds, and fruit pulp, significantly inhibit the growth of different cancer cell lines. Bioactive substances, especially acetogenins, are thought to be responsible for these effects [6, 7, 8]. The potential to increase bioactivity and bioavailability by fermentation of natural compounds has received substantial research [9, 10]. The biological characteristics of compounds can be considerably impacted by the production of bioactive metabolites and modifications to already-existing molecules during fermentation processes [11]. As a result, there is significant potential in examining how fermentation affects *Annona squamosa* seed polysaccharides (ASSPs) and how this affects cancer cell growth. By examining how fermentation processes can improve the antiproliferation activities of ASSPs, this research sets out on a fascinating route. The study specifically targets the human MCF-7 breast cancer cells and the rhabdomyosarcoma cells, with the Vero cell line from an African green monkey's kidney for selectivity. The goal of this study is to evaluate the potential synergistic effects of fermentation on ASSPs to better understand their improved antiproliferative properties and their potential to be a useful addition to the treatment options available for cancer.

Received January 26th, 2024 / Revised April 18th, 2024 / Accepted May 21st, 2024
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Methods

The gathering and naming of *Annona squamosa* fruits

Between June and October of that year, fresh and fully developed sugar apple apples were picked from a garden at Ota-Efun, Olorunda Local Government of Osogbo, in Osun State, Nigeria. The fruits were verified through comparison with pertinent voucher specimens at the Obafemi Awolowo University Herbarium located in the Department of Botany in Ile-Ife, Nigeria, where the specimen copy was submitted and given a voucher number (IFE-17805).

Reagents and chemicals

Every chemical and reagent utilized in this study was of analytical quality and was purchased from various sources.

Processing and fermenting *A. squamosa* seeds

After collecting the fully ripe and tender mature fruits of A. squamosa, the seeds were split into two parts and stored in sterile receptacles. Both fermented and unfermented seeds were treated as previously described [12]. Both fermented and unfermented seeds' cotyledons were taken off of the seed coat and ground up separately in a Warring Blender. The cotyledon and seed coat powder were defatted with n-hexane, then the organic compounds were extracted for 24 hours with 80% (v/v) ethyl alcohol, and the residues were air-dried.

The process of making polysaccharides

The approach used to extract the polysaccharides was based on earlier techniques [13], as shown in Dare and Oyedapo [14]. The precipitates were labelled using the acronyms FSCP, USCP FCP, and UCP for fermented seed coat polysaccharide, unfermented seed coat polysaccharide, fermented cotyledon polysaccharide, and unfermented cotyledon polysaccharide respectively. They were then stored in the refrigerator for use in future studies.

HPLC analysis of the polysaccharides

The composition of the extracted polysaccharides was analysed by the HPLC method [15] and previously reported [14].

Analysing the polysaccharides' ability to prevent the proliferation of cancer cell lines

Using the MTT viability/cytotoxicity assay, the antiproliferative activity of the polysaccharides was examined in human breast cancer cell lines (MCF-7) and rhabdomyosarcoma cell lines (RD) to determine the rate at which various concentrations of the polysaccharides were able to kill the cancer cell lines. Vero cell line, kidney cells from African green monkeys, was used for the evaluation of the selectivity of the polysaccharides. The cell lines were obtained from Centres for Disease Control (CDC), Atlanta, Georgia, USA and made available at WHO National Polio Laboratory, University College Hospital in Ibadan of Oyo State, Nigeria.

Culturing and passaging/sub-culturing of the cells

Cells were cultured in fresh Dulbecco's Modified Eagle Medium (DMEM) mixed with supplements, viz: 0.07% $NaHCO_3$, 10% (v/v), Foetal Bovine Serum (FBS Bioclot, Lot No: 07310), 2 mM L-glutamine, 100 units/ml of penicillin, 100 mg/ml of streptomycin, and 1% non-essential amino acids and vitamin solution. Cultures were kept in a humidified environment with 5% CO_2 at 37 ^{O}C and passaged twice weekly. Cultures that have reached 80–90% confluency as confirmed under the microscope were used for subsequent steps. Cell suspension (100 µl) was separately seeded into 96-well plates ($4x10^5$ cells/well each for MCF-7, RD and Vero) and incubated (in a humidified atmosphere with 5% CO_2 at 37 ^{O}C) for 24 hr for the cells to adhere to the plate.

Treatment of cell cultures

After 24 hr, the growth medium was carefully aspirated out of individual wells and replaced with 200 µl of medium containing varying concentrations (0, 3.125, 6.25, 12.5, 25, 50, and 100 µg/ml) of the polysaccharides and vincristine (vincristine sulphate injection, manufactured by Cipla), and complete DMEM medium was used for the negative control. The treatment was carried out in triplicates for each concentration applied on MCF-7, RD, and Vero cell lines. Plates were kept in an incubator at 37 ^{O}C under a humidified 5% CO_2 atmosphere. After 72 hours, the plates were brought out, and viewed under an inverted microscope (ZEISS Primo Vert) at x100 objective, and the photomicrographs were taken. The cytopathic effect (CPE) scoring was carried out by examining the morphology of the cells under the microscope at a magnification of x100, checking the degree of disruption or destruction of the cells' morphology.

MTT viability assay

The MTT [3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was employed to assess the antiproliferative effect of the polysaccharides and vincristine on MCF-7 and RD cell lines as previously described by Rahman and Sarkar [16]. To allow for MTT cleavage, the polysaccharides and vincristine applied to each well were removed, and 25 µl of MTT (2 mg/ml in PBS) was applied to the individual well. The plates were kept in the incubator and the dark at 37 ^{O}C for 2 hours. After 2 hours, the MTT solution was aspirated off the cells and the purple insoluble formazan crystals formed were solubilised by the addition of 125 µl DMSO. The contents of the plates were mixed on a plate shaker for 15 minutes and the extent of MTT reduction was spectrophotometrically measured at 490 nm on a Multiscan 347 MTX lab spectrophotometer.

The formula below was used to compute the percentage of cytotoxicity or suppression of cell growth:

$$\text{Percentage cytotoxicity} = \left(\frac{A_B - A_T}{A_B}\right) \times 100$$

Where, A_T = Absorbance of treated cells (drug), A_B = Absorbance of negative control (only media)

For each of the cell lines, IC_{50} values—the extract concentration that causes 50% inhibition of untreated cells—were established.

Selectivity Index (SI)

The selectivity index (SI) was computed using a typical kidney epithelial cell line from African green monkeys (Vero). The selectivity index (SI), i.e., the cytotoxic selectivity (i.e., drug safety) for polysaccharides against cancer cells as opposed to normal cells, was derived as the proportion of the cytotoxic effect on the Vero cell line to the cytotoxic effect on cancer (RD and MCF-7) cell lines from the following formula:

$$\text{Selectivity Index} = \frac{IC_{50}\text{calculated for normal cells}}{IC_{50}\text{calculated for cancer cells}}$$

High selectivity was defined as SI values greater than 2 [17].

Statistical Evaluation

The results were presented as Mean ± standard error of the mean (SEM), for n ≥ 3 measurements. GraphPad Prism 5 was used to do a one-way ANOVA and post-test of Tukey multiple comparisons to identify differences between the mean values of the control and treated groups. If $p < 0.05$, differences were deemed significant.

Results

The cytopathic effect (CPE) of the polysaccharides on the RD cell line is presented in Table 1. Plate 1 is the representative photomicrographs of the 0% and 100% CPE of the RD cell line treated with the extracted polysaccharides. Figure 1 is the presentation of the percentage cytotoxicity of the polysaccharides on the RD cell line with the vincristine standard. The percentage of cytotoxicity demonstrated a dose-dependent response, increasing with higher concentrations of the polysaccharides and vincristine. The calculated IC_{50} values for FSCP, USCP, UCP, and FCP on RD are shown in Table 2.

Table 1 Cytopathic Effect (CPE) Scoring for Rhabdomyosarcoma (RD) Cell Line

Concentration (µg/ml)	FSCP	USCP	FCP	UCP	Vincristine
0.000	–	–	–	–	–
3.125	–	–	–	–	2+
6.250	–	–	–	–	3+
12.500	–	–	–	–	3+
25.000	–	–	+	–	4+
50.000	2+	2+	4+	2+	4+
100.000	4+	4+	4+	4+	4+

Key: 4+ was 100% CPE (complete damage and death of the cell population), 3+ was 75% CPE (75% damage/death of cell population), 2+ was 50% CPE (50% damage/death of the cell population), + is 25% CPE (25% damage/death of the cell population), - was 0% CPE (no damage/death)
FSCP, USCP, FCP, and UCP were the acronyms for fermented seed coat polysaccharide, unfermented seed coat polysaccharide, fermented cotyledon polysaccharide, and unfermented cotyledon polysaccharide respectively

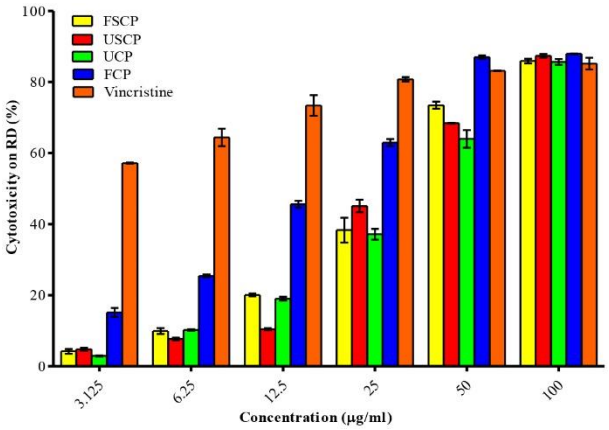
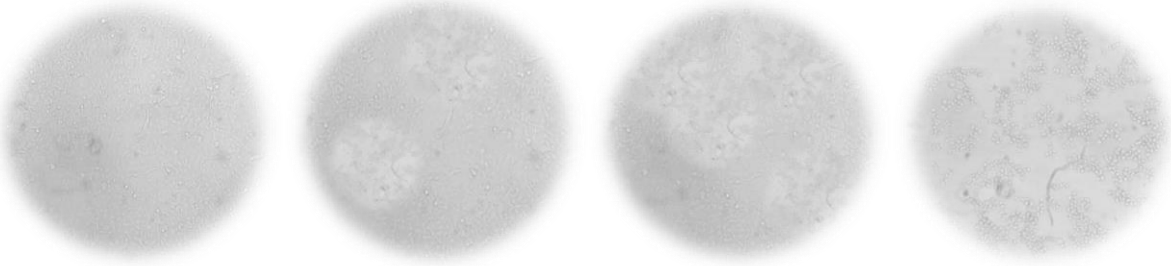


Fig. 1 Cytotoxicity on Rhabdomyosarcoma (RD) Cell Line
Each value stood for Mean ± SEM, n = triplicates
FSCP stands for fermented seed coat polysaccharides, USCP for unfermented seed coat polysaccharides, FCP for fermented cotyledon polysaccharides, and UCP for unfermented cotyledon polysaccharides



0% CPE 25% CPE 50% CPE 100% CPE

Plate 1 Morphology of RD Cancer Cell Line Treated with Polysaccharides (0% and 100% CPE) at x100

Table 2 IC_{50} Values for Viability Test on RD Cell Line

Sample	IC_{50} (µg/ml)
FSCP	$45.91 \pm 0.78^{c,d}$
USCP	47.00 ± 0.44^{c}
FCP	$27.10 \pm 0.61^{a,b,d}$
UCP	$49.23 \pm 0.59^{a,c}$

Each value stood for Mean ± SEM, n = triplicates. The statistical significance was reached at $p < 0.05$ for the values with alphabet superscripts, [a] compared FSCP to the rest, [b] compared USCP to the rest, [c] compared FCP to the rest, [d] compared UCP to the rest.
FSCP, USCP, FCP, and UCP were the acronyms for fermented seed coat polysaccharide, unfermented seed coat polysaccharide, fermented cotyledon polysaccharide, and unfermented cotyledon polysaccharide respectively

The cytopathic effect (CPE) of the polysaccharides on the MCF-7 cancer cell line revealed that the four polysaccharides elicited various cytotoxic effects on the cell line at the concentrations used (Table 3). In Plate 2 are the representative photomicrographs of various degrees of CPE in MCF-7 on treatment with the polysaccharides. Figure 2 shows the dose-dependent response of the percentage cytotoxicity of the polysaccharides on MCF-7 with standard vincristine. The median lethal concentration (IC_{50}) of the polysaccharides on MCF-7 is in Table 4.

Table 3 Cytopathic Effect (CPE) Scoring for MCF-7

Concentration (µg/ml)	FSCP	USCP	FCP	UCP	Vincristine
0.000	–	–	–	–	–
3.125	–	–	–	–	2+
6.250	–	–	–	–	3+
12.500	–	–	–	–	3+
25.000	–	–	+	–	4+
50.000	2+	+	4+	–	4+
100.00	4+	4+	4+	4+	4+

Key: 4+ was 100% CPE (complete damage/death of the cell population), 3+ was 75% CPE (75% damage/death of cell population), 2+ was 50% CPE (50% damage/death of the cell population), + was 25% CPE (25% damage/death of the cell population), - is 0% CPE (no damage/death)
FSCP, USCP, FCP, and UCP were the acronyms for fermented seed coat polysaccharide, unfermented seed coat polysaccharide, fermented cotyledon polysaccharide, and unfermented cotyledon polysaccharide respectively

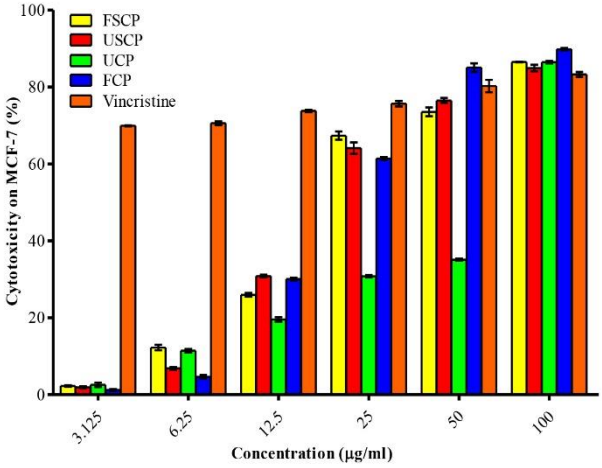


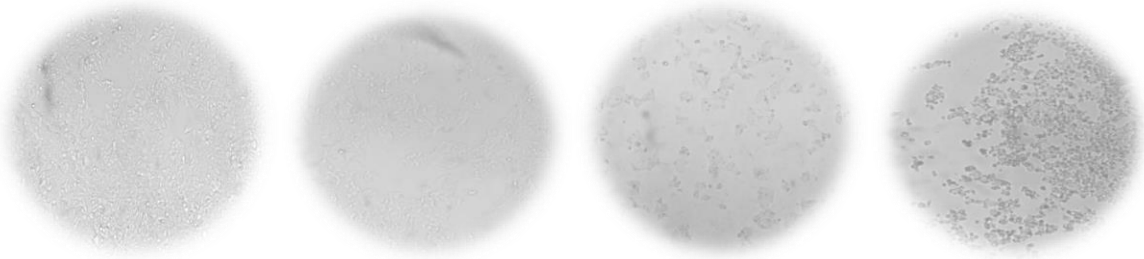
Fig. 2 Cytotoxicity on Breast Cancer (MCF-7) Cell Line
Each value stood for mean ± SEM, n = triplicates
FSCP, USCP, FCP, and UCP were the acronyms for fermented seed coat polysaccharide, unfermented seed coat polysaccharide, fermented cotyledon polysaccharide, and unfermented cotyledon polysaccharide respectively

Table 4 IC_{50} Values for Viability Test on MCF-7 Cell Line

Sample	IC_{50} (µg/ml)
FSCP	$39.34 \pm 0.53^{c,d}$
USCP	$39.88 \pm 0.50^{c,d}$
FCP	$37.91 \pm 0.35^{b,d}$
UCP	$57.01 \pm 0.06^{a,b,c}$

Each value stood for Mean ± SEM, n = triplicates. Statistical significance was reached at $p < 0.05$ for the values with alphabet superscripts, [a] compared FSCP to the rest, [b] compared USCP to the rest, [c] compared FCP to the rest, [d] compared UCP to the rest.
FSCP, USCP, FCP, and UCP were the acronyms for fermented seed coat polysaccharide, unfermented seed coat polysaccharide, fermented cotyledon polysaccharide, and unfermented cotyledon polysaccharide respectively

As shown in Figure 3, the percentage of polysaccharides that inhibited the Vero cell line's proliferation was minimal. On Vero cells, the polysaccharides had extremely high IC_{50} values (Table 5). The selectivity indices (SI) calculated as a proportionate of the IC_{50} values of the polysaccharides on normal Vero cells to the IC_{50} values on MCF-7 and RD (Table 6) were more than one in the four polysaccharides.



0% CPE 25% CPE 50% CPE 100% CPE

Plate 2 Morphology of MCF-7 Cell Line Treated with Polysaccharides (0, 25%, 50% and 100% CPE) at x100

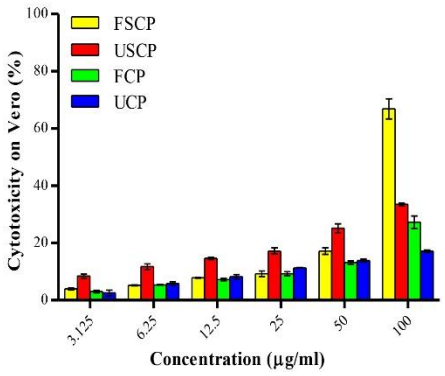


Fig. 3 Cytotoxicity on Vero Cell Line
Each value stood for mean ± SEM, n = triplicates
FSCP, USCP, FCP, and UCP were the acronyms for fermented seed coat polysaccharide, unfermented seed coat polysaccharide, fermented cotyledon polysaccharide, and unfermented cotyledon polysaccharide respectively

Table 5 IC_{50} Values for Viability Test on Vero Cell Line

Sample	IC_{50} (µg/ml)
FSCP	160.88 ± 2.62[b,c,d]
USCP	83.76 ± 4.25[a,c,d]
FCP	218.12 ± 0.74[a,b,d]
UCP	339.04 ± 4.54[a,b,c]

Each value stood for Mean ± SEM, n = triplicates. Statistical significance was reached at $p < 0.05$ for the values with alphabet superscript, [a] compared FSCP to the rest, [b] compared USCP to the rest, [c] compared FCP to the rest, [d] compared UCP to the rest.
FSCP, USCP, FCP, and UCP were the acronyms for fermented seed coat polysaccharide, unfermented seed coat polysaccharide, fermented cotyledon polysaccharide, and unfermented cotyledon polysaccharide respectively

Table 6 Selectivity Indices with Vero Cell Line

	Selectivity Index	
Sample	RD	MCF-7
FSCP	3.50 ± 0.03	4.09 ± 0.03
USCP	1.78 ± 0.01	2.10 ± 0.01
FCP	8.18 ± 0.22	5.76 ± 0.03
UCP	6.89 ± 0.04	5.95 ± 0.01

Each value stood for Mean ± SEM, n = triplicates.
FSCP, USCP, FCP, and UCP were the acronyms for fermented seed coat polysaccharide, unfermented seed coat polysaccharide, fermented cotyledon polysaccharide, and unfermented cotyledon polysaccharide respectively

Discussion

As it was previously reported in our study [14], unfermented seed coat and cotyledon had higher yields than the fermented ones. Also, the concentrations of total soluble sugar, hexosamines, and uronic acids were higher in the unfermented seed coat and cotyledon than in their fermented counterpart. The increase in these parameters in unfermented compared to fermented could be a result of an increase in α-amylase and α-glucosidase activities which have been reported to accompany fermentation [18]. The MTT viability assay was carried out on RD and MCF-7 using the four polysaccharides to assess their cytotoxic/anti-cancer potentials. The IC_{50} values of the polysaccharides on the RD (Table 2) showed FCP to be the most potent with the lowest IC_{50} value (27.10 ± 0.61 µg/ml). The four polysaccharides elicited no cytopathic effect on the rhabdomyosarcoma (RD) cell line at concentrations below 25.00 µg/ml (Table 1).

Both the polysaccharides and regular vincristine were evaluated for their cytopathic effect. A cytotoxic drug can produce structural alterations in cancer cells known as cytopathic effect (CPE) or cytopathogenic effect (CPE). The cell either dies without lysing as a result of the cytotoxic substance or dies because it cannot divide. The CPE is to blame for both of these impacts. Rounding of the cell, joining of adjacent cells to create syncytia, and the formation of nuclear or cytoplasmic inclusion bodies are common manifestations of CPE. The CPE is expressed in percentage based on the degree of cytotoxicity expressed by the cytotoxicant (a measure of the degree of death within the cell population inferred from the alteration in the shape and form of the cells as a result of the cytotoxic action of the drug, viewed under the microscope).

The most severe form of CPE that results in the breakdown of the cancer cell monolayer is 100% CPE, which is signified by a score of 4+ in the CPE scoring system. In a process known as pyknosis, every cell in the monolayer quickly contracts, thickens, and separates from the plate. The 75% (denoted by 3+ in CPE scoring) and 50% (denoted by 2+ in CPE scoring) CPEs are the subtotal elimination of the cancer cell, which is generally evidenced by the detachment of some but not all of the cells within the monolayer. The 25% CPE (denoted by + in CPE scoring) is the initial stage of cytotoxicity where spreading occurs at localised cell centres known as foci, causing a localised attack of the cancer cell monolayer.

In morphology, RD is normally a fibroblastic (or fibroblast-like) cell line which is bipolar or multipolar, has an elongated/spindle shape, is large and multinucleated, and grows attached to a substrate. Microscopic visualisation of the RD cells after treatment showed that the cells had lost their normal morphology; became rounded up at 100% CPE, detached from the plate, and the monolayer was completely lost (Plate 1) while the cells of the control (untreated) maintained their original morphology.

Due to fluid accumulating between the cell monolayer and the culture plate, the MCF-7 cell line's monolayers develop dome-like structures with an epithelial-like appearance. Polygonal and having more uniform proportions, epithelial-like cells form distinct patches that are coupled to a substrate. The cells started to separate from the culture plate's base under a microscope, which indicated that the extracellular matrix had been disrupted and that cell-to-cell contact had been inhibited (Plate 2). There was no cytotoxicity noticed in the four polysaccharides until 50 µg/ml and 100 µg/ml (Table 3). Analysis with MTT indicated almost 90% cytotoxic action of the tested polysaccharides on MCF-7 and RD cell

lines at the highest concentration used (100 µg/ml. Additionally, microscopic comparisons of cells given treatment with polysaccharides and untreated controls revealed cytostatic effects since active cellular expansion (growth) was observed in controls but suppressed in treated cells. Fermented cotyledon polysaccharides (FCP) demonstrated the highest cytopathic and cytotoxic effects on both the RD and MCF-7 cell lines.

There have been claims that several plant-derived polysaccharides have cytotoxic or antitumour properties. Wang *et al.* [19] demonstrated the antitumour activities of two polysaccharides fractions from *Dendrobium nobile*, DNP-W1 and DNP-W3, against sarcoma 180 *in vivo*, with the inhibition ratio of 65.3% and 61.2% respectively. The two fractions also exhibited good protection against the growth of HL-60 cells. Meng *et al.* [20] reported that mice sarcoma 180 was seriously inhibited by *Dendrobium candidum* polysaccharide (DP).

FCP had the lowest IC_{50} value (37.91 ± 0.35 µg/ml) which was significantly ($p < 0.05$) dissimilar to those of UCP, USCP, and FCSP (Table 4.23). The IC_{50} value shows the inhibition concentration at which only 50% of the cells are viable. According to the National Cancer Institute (NCI) guidelines, $IC_{50} < 100$ µg/ml is considered active [21, 22]. Thus, all four polysaccharides could be considered active on both RD and MCF-7 according to National Cancer Institute (NCI) guidelines. Among all the four polysaccharides tested, FCP displayed the most cytotoxic potential, especially with the RD with an IC_{50} value of 27.10 ± 0.61 µg/ml. The IC_{50} values of the polysaccharides except unfermented seed coat polysaccharides (USCP) on Vero were significantly higher than 100 µg/ml (Table 5), showing their low cytotoxic effects on the Vero cells.

It is crucial to understand that the ability to selectively kill cancer cells is the primary characteristic of a clinically effective anticancer treatment; without selectivity, cytotoxic potency and the pharmacological target are useless parameters [23]. This is a drawback to using several chemotherapeutic drugs. So, when looking for possibilities for cancer treatment, selective toxicity must be taken into account [24]. The selectivity index (SI) is a ratio that measures the window between cytotoxicity on regular cells and cytotoxicity on cancer cells. Theoretically, medicine would be more efficient and safer when used *in vivo* to treat a specific cancer case if the SI ratio was larger. The ideal drug would have a high SI value, be cytotoxic to normal cells at extremely elevated concentrations, and exhibit cytotoxic activity on cancer cells at very low concentrations, eradicating the target cancer cells at concentrations well below those that rendered them cytotoxic to normal cells. A metric that is widely accepted for expressing a compound's *in vitro* effectiveness in inhibiting cancer cell proliferation is its selectivity index [25].

An SI value less than 2.0 indicates the general toxicity of the compound, and the greater the SI value of a sample, the more selective it is [26]. In the hunt for anticancer medication, it's been documented in the literature that a selectivity index with a value more than or equal to 2.0 is exceptional [17]. This value implies that the compounds are twice or more cytotoxic to the tumour cell lines as compared with the normal cell line. The selectivity index is of greater interest when the SI values are greater than three [27].

The selectivity indices of the purified polysaccharides using the Vero cell line were higher than 2.0 for both RD and MCF-7 (Table 6) except for USCP on RD. From the selectivity studies, UCP had a SI value of 8.0 on RD and 5.8 on MCF-7. This means that UCP was eight times more toxic to RD and six times more toxic to MCF-7 compared to normal cells (Vero), an indication that its cytotoxic activity is selective for the cancer cells under investigation. Similarly, FCP displayed an SI value of 6.9 on RD and 5.9 on MCF-7, suggesting that this polysaccharide has high selectivity for cancer cells.

It has also been demonstrated that polysaccharides' anti-tumour properties depend in part on their structural makeup, and studies have proven that modified polysaccharides depicted altered structures and ultimately enhancement of the anti-cancer efficacy of the polysaccharides [28, 29]. The modification effect that could result from fermentation may probably be responsible for the significantly better bioactivity exhibited by fermented cotyledon polysaccharides (FCP) in this study.

Conclusion

The results of this study established the anti-proliferation properties of the purified polysaccharides (FCP, UCP, FSCP, USCP) from *Annona squamosa* cotyledon and seed coat. The findings of this study provided basic information on the anti-cancer potentials of polysaccharides from fermented and unfermented *A. squamosa* cotyledon and coat and the enhancing role of fermentation in the bioactivities of phytochemicals. Additional investigation is necessary to confirm the mechanism of the antiproliferative action and the full structures of these polysaccharides.

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