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Research Article

Activities of Polysaccharides Derived from Commiphora Gileadensis on Colorectal Cancer Cell Lines

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Abstract

Background: CRC is a common malignancy leading to cause mortality globally. *Commiphora gileadensis* was used to treat respiratory infections, neuralgic pain, wounds and arthritis.
Methods: *Commiphora gileadensis* was harvested; then polysaccharides were extracted. The antiproliferative activity was assessed on two colorectal cancer cell lines; then, an apoptosis assay was conducted to determine cell death. Polysaccharides with antiproliferative effects were digested and separated.
Results: *Commiphora gileadensis* separation confirmed the presence polysaccharides that showed antiproliferative activities on SW480 and SW620 with IC50 13.15 µg/mL and 32.02 µg/mL, respectively. Cell death was activated via apopstosis on SW480, with some resistance on WS620 cell line. Discussion: The cytotoxic effects of*Commiphora gileadensis* polysaccharides on SW480 and SW620 were significant as it activated the cellular apoptosis pathway.
Conclusion: *Commiphora gileadensis* polysaccharide extracts induce cellular apoptosis on CRC cell line with potent antiproliferative activity.

INTRODUCTION

Carbohydrates are widely recognised as the most predominant biomolecule in nature and are the primary fuel for energy production. Based on the chain length, carbohydrates can be classified into monosaccharides, oligosaccharides, and polysaccharides (Nelson et al., 2008). Monosaccharide is the simplest carbohydrate type, which is divided into polyhydroxy aldehydes and polyhydroxy ketones based on the position of their carbonyl group (C= O)(Chaplin & Kennedy, 1986). Mono-saccharides can be found in linear or ring form and contain chiral carbon (Chaudhary et al., 2022). Chiral carbons help determine the monosaccharide isomerisa-tion concerning the hydroxyl group location, which defines the monosaccharide structure as either dextrorotary (D) or levorotary (L) (Bertozzi & Rabuka, 2009; Finelli, 2019). Oligosaccharides are made up of ring form monosaccharides forming a polymer structure comprising 2 to 10 monosaccharides linked together via glycosidic linkage, either α or β glycosidic linkages (Boehm & Stahl, 2003). Polysaccharides are made up of long unbranched or branched chains of monosaccha rides linked together by α or β glycosidic bonds (Aspinall, 2014; Niaz et al., 2020). In addition, poly-saccharides can be classified into two categories based on their monosaccharide molecules: heteropolysaccha rides and homopolysaccharides (Nasrollahzadeh et al., 2021). Homopolysaccharides are composed of a single type of monosaccharide that repeats along the chain. On the other hand, heteropolysaccharides consist of more than one type of monosaccharide that repeats, such as glycosaminoglycan (Stanley et al., 2009; X. Zhang et al., 2019).

Cancer is an unregulated cellular growth disorder that can be benign or malignant. Colorectal cancer (CRC) is

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a malignant tumour caused by unregulated colon or rectal cell growth (Mattiuzzi et al., 2019). As a common malignancy, CRC is the third leading cause of death worldwide (Rawla et al., 2019), as Saudi Arabia's second most reported cancer is CRC (Almatroudi , 2020). It ranks first in men and second in women after breast cancer (Khoja et al., 2018). In advanced-stage cancer patients, CRC symptoms include weight loss, tiredness, bowel motility abnormalities, and colon haemorrhage. About 95% of CRC incidents are adeno carcinomas , with gastrointestinal stromal tumours, lymphomas, sarcomas, and carcinoid tumours (Mattiuzzi et al., 2019).

CRC has two risk factors, genetic and environmental, which significantly influence its development and progression (Shanehbandi et al., 2021). The genetic predisposition factors are sporadic and hereditary, which could be further divided into hereditary adenomatous polyposis and non-polyposis (Arvelo et al., 2015). Sporadic CRCs afflict 70-year-olds, accounting for 85% of CRC cases (Dekker et al., 2019; Yamagishi et al., 2016). Sporadic CRC has no germline mutation or inheritance pattern. Hereditary CRC is a group of illnesses with a genetic mutation that causes a clinically unique phenotype (Pabón & Babiker, 2022). On the other hand, environmental factors, excessive alcohol consumption, smoking, obesity, sedentary behaviour, processed red meat, a high-fat, low-fibre diet, and other lifestyle variables increase the risk of CRC.

CRC treatment aims to improve health and survival, including surgery, radiation, and systemic chemotherapy (Knowlton et al., 2013; Van Cutsem et al., 2017). Chemotherapy is a tumour therapy that reduces cancer cell growth by blocking cell division or damaging cellular deoxyribonucleic acid (DNA) (Florea & Büsselberg , 2011). For example, 5-fluorouracil (5-FU) is an antimetabolite that substitutes fluorine at the uracil C-5 position with hydrogen (Vodenkova et al., 2020). 5-FU is the usual treatment for CRC. CRC patients have significant adverse effects, such as fever, stomatitis, vomiting, diarrhoea, anaemia, leukopenia, thrombocytopenia, neuropathy, and skin rash were also reported (Chionh et al., 2017; Jessica Latchman & Ann Guastella, 2014; Vodenkova et al., 2020; L. Zhang et al., 2018). Chemotherapeutics' key drawbacks include cancer recurrence, limited specificity, drug resistance, restricting the use of antiproliferative agents, and lowering patient's overall health and quality of life (Choudhari et al., 2020). Chemotherapy's significant side effects may contribute to increased mortality rate; therefore, innovative antiproliferative medications are developed based on their tolerance and safety, as shown in various natural products (Alshehri, 2020; Herrera-Calderon et al., 2020).

Commiphora gileadensis (C. gileadensis), a shrub species belonging to the Commiphora genus, is indigenous to Egypt, Saudi Arabia, Yemen, Oman, and Syria. It is also referred to as the Arabian balsam tree (Alsherif , 2019). Throughout history, the plant has gained remarkable medicinal attributes ascribed to its fluid, wood, bark, and seeds. It is harvested for its gum or sap to treat respiratory infections and relieve rheumatic and neuralgic pains. Resin-based treatments are utilised in traditional medicine to treat disorders such as arthritis, wounds (Alhazmi et al., 2022), gastrointestinal distress, obesity, pain, and parasitic infections (Al-Harbi et al., 1997). Chloroform extracts of fresh stems demonstrated enhanced wound healing in rats (Althurwi et al., 2022) and anti-inflammatory and antibacterial properties (Alhazmi et al., 2022).

Leaf and twig aqueous extracts successfully adjusted the glucose levels of hypercholesterolemic diabetic rats, nearly restoring all histopathological and biochemical parameters (El Rabey et al., 2020a). The ethanolic extracts of the sap have been shown to effectively induce apoptosis in both immortalised and transformed human epidermal cell lines (Wineman et al., 2015). This study aims to explore the antiproliferative properties of polysaccharides derived from C. gileadensis on CRC cell lines. These findings would broaden the potential of using polysaccharides derived from natural products as pharmaceutical agents.

MATERIALS AND METHODS

C. gileadensis Collection

In July 2020, specimens of the C. gileadensis tree were gathered from the Alaab Valley in the western Makkah region of Saudi Arabia.

C. gileadensis Polysaccharides Extraction

The polysaccharides extraction procedure was previously published (Aldairi et al., 2018). Briefly, the freshly harvested stem of C. gileadensis was dried out using an oven vacuum at 50 ○C under 60 mmHg for 8 hours. The dried C. gileadensis was crushed and powdered finely. In order to obtain carbohydrates from the sample, this process should follow the removal of lipids and protein residues from the sample (Kim et al., 1996; Urbanova & Adams, 1970)

C. gileadensis crude (50 g) was submerged in acetone (EMPARTA® grade, Merck, USA) (200 mL) and incubated for 72 hours; then, acetone was discarded, and plants left on foil to allow the removal of acetone residues for 48 hours at room temperature. Following the removal of lipids, protein residues were removed from the C. gileadensis using a broad-spectrum alcalase en -zyme derived from Bacillus licheniformi (Merck, USA) for 48 hours.

After removing protein residues by precipitation, the supernatant was retained. Polysaccharides were precipitated from the supernatant using absolute ethanol (EMPARTA® grade, Merck, USA) and 2.5 M NaCl. Thus, it would allow highly negatively charged polysaccharides to be extracted. Finally, the polysaccharide extract was retained using lyophilisation against dH2O,

and then stored at -80 ℃ for further analysis.

Cell lines maintenance

RPMI 1640 Medium, GlutaMAX ™ (Gibco, USA), 10% heat-inactivated foetal bovine serum (FBS) (Gibco, USA), penicillin 100 units/mL, and streptomycin 100 µg /mL (Gibco, USA) were used to seed SW480 and SW620 cell lines. Cell lines were cultured in a T75 cm2 flask at 37 ○C under 5% CO2 humidity.

Cell viability assay

In a 96-well plate, cells were seeded at 3 × 103 cells/well and incubated in 5 % CO2 at 37 ○C for 24 h. Afterwards, 100 µL of the drug was added to a 96-well plate of SW480 and SW620 in a triplicate set with a maximum dose concentration of 500 µg/mL (0, 15.62, 31.25, 62.5, 125, 250, 500). After that, cells were incubated at 37 ℃ with 5% CO2 for 96 h.

Apoptosis assay

Following treatment, cells were briefly analysed using an Annexin V-Alexafluor 288 (AF488)/PI Apoptosis Assay Kit (Thermo Fisher Scientific, USA). The cells were harvested following treatment and washed three times with cold PBS. The cell precipitate was reconstituted in 100 µL of 1× Annexin V (AV)-binding buffer. Each 100 µL of SW620 cell suspension was stained with AV-AF488 (5 µL) and 1 µL of propidium iodide (PI) solution (10 µL) at room temperature for 15 min (in the dark). After incubation, 400 µL of the binding buffer was added to the stained cells and analysed using NovoCyte 3000 flow cytometer (Agilent, USA). The experiments were carried out in triplicate and represented as mean ± SD of the different apoptosis stages. Live cells (AV-AF488neg/ PIneg), Early Apoptosis (EA; AV-AF488pos/ PIneg), Late Apoptosis (LA; AV-AF488pos/ PIpos), and Dead cells (AV-AF488neg/ PIpos) (Aldairi et al., 2018).

Polysaccharides Digestion

200 µg of the provided polysaccharides were used for enzymatic digestions with chondroitinase ABC and Heparinase I-III. Chondroitinase -ABC catalyses the degradation of polysaccharides containing (1 □4) β-D-hexosamine, and (1 □3) β-D-glucuronic aicd or (1 □3) α-L-iduronic acid linkages to disaccharides containing 4-deoxy-β-D-gluc-4-enuronosyl groups. On the other hand, heparinases I-III are combined to cleave heparin and heparan Sulfate into disaccharides. The disaccha-rides produced were collected between each digestion with an MWCO filter (10 kDa) (Lawrence et al., 2008).

Strong Anion Exchange-High-performance Liquid Chromatography (SAX-HPLC)

An Agilent system was used to perform SAX-HPLC, utilising a 4.6×250 mm Spherisorb analytical column with particle size 5 µm (Waters, USA). The solvent A consists of a solution containing 2.5 mM Na3PO4, with a pH of 3.5. Solvent B consists of a concentration of 2.5 mM of Na3PO4 and 1.2 M NaCl with a pH of 3.5. The flow rate is 1.0 mL/min—injection volume of each sample: 10 µL , 90 µL . Disaccharide standards were purchased from Dextra Laboratories, USA, to identify each disaccharide based on elution time and calibration (Lawrence et al., 2008).

Statistical analysis

Statistical analysis was performed with GraphPad Prism 9.0 (La Jolla, CA, USA). The determination of the half-maximal inhibitory concentration (IC50) value was done after getting the average cell values of triplicates by nonlinear regression. The data were normalised from the highest value of 100% to the lowest value of 0% to create a dose-response curve. Apoptosis assay results were determined by normalising data and using a fit curve with nonlinear regression (inhibitor vs normalised response).

RESULTS

Cell viability assay

The antiproliferative activity of C. gileadensis polysaccharides was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against two cell lines, namely, SW480 and SW620. Polysaccharide extracts showed IC50 13.15 µg/mL against SW480 and 32.02 µg/mL against SW620 cell lines (Table 1). The growth inhibition of each cell line is shown in (Figure 1).

Table 1: Calculated values of IC50 on SW480 and SW620 CRC cell lines.

Drug	IC50	
	SW480	SW620
C. gileadensis Polysaccharides extracts (µg/mL)	13.15	32.02
5-FU (µM)	2.748	2.082

Apoptosis Assay

CRC cell line SW480 showed significant increases in both early and late apoptotic areas (Annexin V+/PI+) when treated with different doses of C. gileadensis polysaccharides extracts after 24 hours of incubation. The results shown in Figure 2 indicated that apoptosis is the most likely cause of the cytotoxicity following the treatment by C. gileadensis polysaccharides (Figure 2). Additional doses, such as 2× and 4× the IC50, were used to address the doses, and the antiproliferative effects were dose-dependent . On the other hand, the SW620 cell line showed notable increases in the late apoptotic region when treated with the IC50; however, the cells showed more potent effects toward the apoptotic region when treated with 2× concentration of the IC50 of C.gileadensis polysaccharides extracts following 24 h incubation (Figure 3).

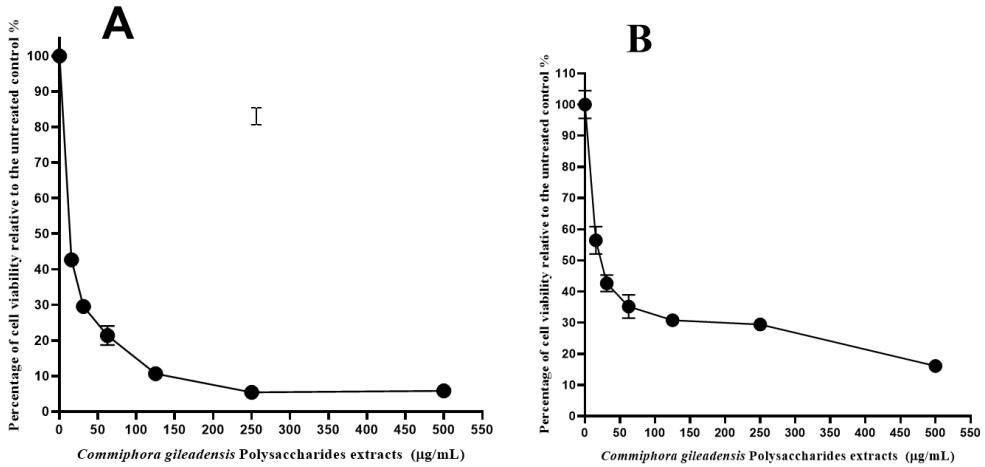

Figure 1: The antiproliferative activities of C. gileadensis polysaccha-ride extract on cancer cell lines. CRC cell lines SW480 (A) and SW620 (B) were treated with rising doses of C. gilead-ensis polysaccharides extracts as determined by MTT assay.

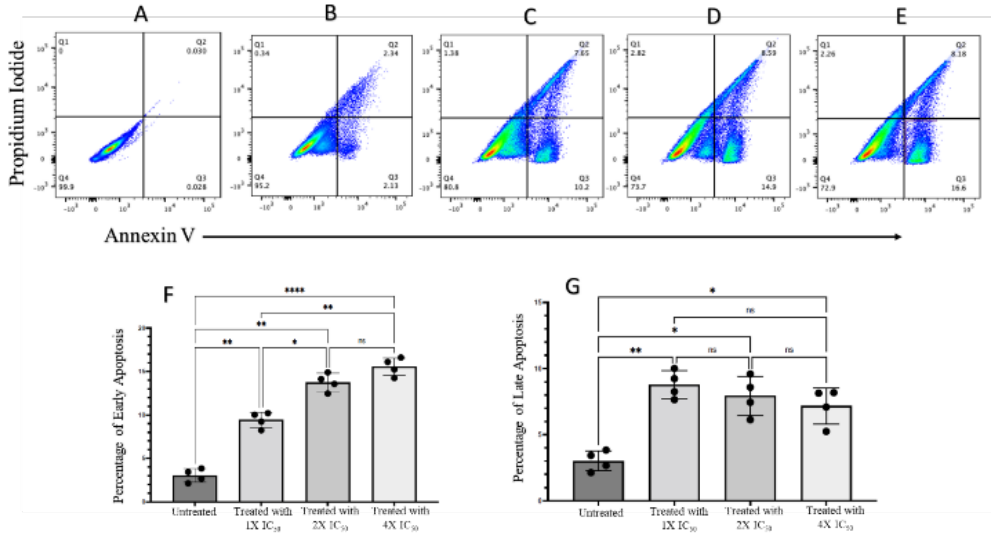

Figure 2: Flow cytometry plot of Annexin V-FITC/Propidium iodide-stained SW480 cell line treated with several doses of C. gileadensis polysaccharides extracts. (A) Showed unstained cells, (B) Control cells, (C) cells treated with C. gileadensis polysaccharides extracts IC50 at a dose of 13.15 µg/mL, (D) cells treated with C. gileadensis Polysaccharides extracts 2× the IC50 at a dose of 26.3 µg/mL, (E) cells treated with C. gileadensis Polysaccharides extracts 4× the IC50 at a dose of 52.6 µg/mL. (F) showed the percentage of early apoptosis, where all doses showed a significant increase in apoptosis. (G) showed the percentage of the late apoptosis, where all doses showed a significant increase in apoptosis.

Disaccharide Analysis by SAX-HPLC

The sample was digested using heparanase I-III (Figure 4) and Chondroitinase ABC (Figure 5) enzymes to break down isolated polysaccharides into disaccharides. Only trace signals were observed compared to the sample standards (Figure 6), and a few additional peaks were observed in the traces. Since they were observed in both digestions, we assume they pre-existed in the sample and are unaffected by enzymatic digestion.

DISCUSSION

CRC is a common form of cancer worldwide (Xi & Xu, 2021). Several treatment regimes were used in treating CRC, for instance, surgical removal of the tumour and chemotherapy (Hossain et al., 2022); however, several side-effects of chemotherapy were reported, for example , cancer recurrence, drug resistance and low specificity (Choudhari et al., 2020), reduced patient's quality of life (Huang et al., 2019). Recent research has shown that natural products, such as Curcumin (Herrero de la Parte et al., 2021), Terminalia catappa (Shanehbandi et al., 2021) and Thymoquinone (Almajali et al., 2021),

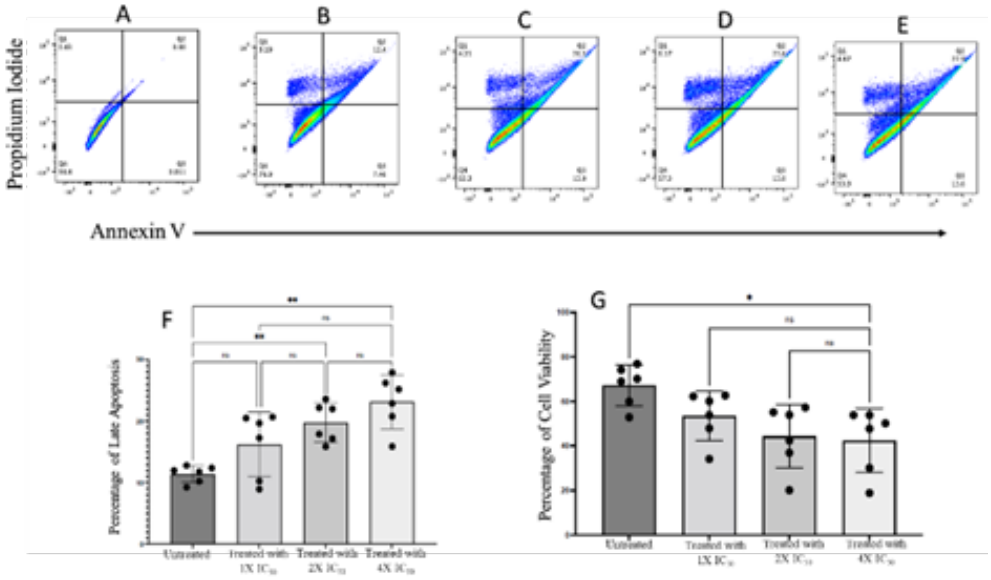

Figure 3: Flow cytometry plot of Annexin V-FITC/Propidium iodide-stained SW620 cell line treated with several doses of C. gil-eadensis Polysaccharides extracts. (A) Showed unstained cells, (B) Control cells, (C) Cells treated with C. gileadensis Polysaccharides extracts with the IC50 value at a dose of 32.02 µg/mL, (D) Cells treated with C. gileadensis Polysac-charides extracts 2× the IC50 value at a dose of 64.04 µg/mL, (E) Cells treated with C. gileadensis Polysaccharides extract 4× the IC50 value at a dose of 128.08 µg/mL. (F) The per-centage of late apoptosis in cells treated with the same IC50 value showed no significant difference over the control cells. In contrast, cells treated with 2× and 4× the IC50 value showed significant differences over the control cells. (G) Showed the percentage of viable cells over 24 h incubation, at the concentration of 128.08 µg/mL, was the only dose showing significant cellular death.

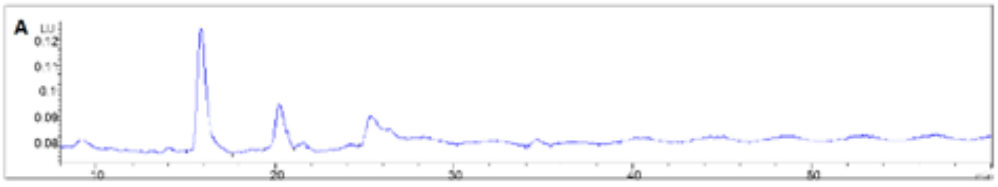

Figure 4: SAX-HPLC of Heparinase I-III materials

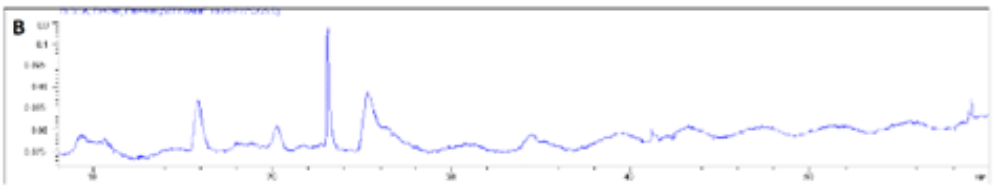

Figure 5: SAX-HPLC of Chondroitinase-ABC materials.

found to possess anticancer properties and have been distinguished as having fewer adverse effects than other chemotherapeutic drugs, could be used as an alternative treatment for CRC. Carbohydrates are considered one of the most prominent nutrients in nature (Nelson et al., 2008), and they exhibit a wide range of chemical compositions, structural diversities, and molecular weight that make them potential candidates for cancer treatment.

Polysaccharides derived from various sources of marine life showed medicinal properties; for instance, a unique glycosaminoglycan structure, known as hybrid heparin/HS, was extracted from shrimp's head Litopenaeus vannamei , exhibiting anticoagulation activities and extended clotting time (Brito et al., 2014), a highly sulphated heparan sulphate-like structure was identified from marine bivalve mollusc known as Nodipecten nodosus , which exhibited a six-fold reduction in anticoagulant activity compared to porcine heparin (Gomes et al., 2010), glycosaminoglycan-like derived marine cockle Cerastoderma edule showed potent antiproliferative activities against leukaemia cell lines (Aldairi et al., 2018), mushroom-derived polysaccha rides, known for their anticancer properties, act as immunomodulators or biological response modifiers, preventing cancer cell growth in animal models (Zong et al., 2012). Since polysaccharides were used as a phar-maceutical agent, which was derived from a wide source of natural prod-

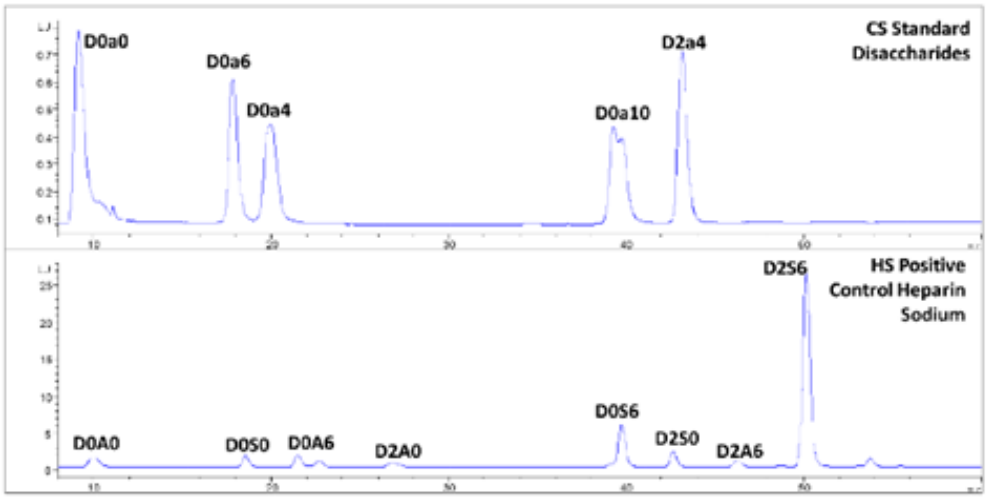

Figure 6: SAX-HPLC of CS standards and labelled heparin digestion products (used as a positive control).

ucts, C. gileadensis was used as a natural source with possible therapeutic tool due to its exceptional medicinal properties, such as antihypertensive (Iluz et al., 2010), antidiabetic (El Rabey et al., 2020b), and antimicrobial activity (Al-Sieni , 2014; Alhazmi et al., 2022).

The current study explored the polysaccharides extract's antiproliferative activity derived from C. gileadensis on two CRC cell lines, which are SW480 and SW620. As SW480 is a primary tumour derived cell (Hewitt et al., 2000), the antiproliferative effects of C. gileadensis polysaccharides showed potent cytotoxic activities with IC50 13.15 µg/ mL. These antiproliferative effects were shown to have significant values toward activating the cellular apoptosis pathway when treated with several doses over 24 h. According to Wang et al., plant extracts derived from Panax notoginseng showed antiproliferative activities on SW480 with IC50 200 µg/mL (Wang et al., 2009) as they used the chemical extractions only by ethanol and n-butanol, were no further biological extraction methods reported. Another plant lectin extract from Phaseolus acutifolius showed antiproliferative effects with IC50 100 µg/mL as the maximum concentration on SW480, while plant lectins did not show any activities on other cell lines (Valadez-Vega et al., 2014). Vaccinium meridionale berries extracts were applied on SW480 with an IC50 536 µg/mL (Zapata Vahos et al., 2019). Another study on green and black tea leaves was estimated to have a cytotoxic effect on SW480, where the black tea had an IC50 of 26.3 µg/mL and green tea showed an IC50 of 36 µg/mL (Zapata-Vahos et al., 2015). However, the determination of the IC50 used a sulforhodamine assay, which depends on a particular protein obtained from cells, and there is no further biological evidence of cellular death. Villota et al. claimed to have cytotoxic effects of green coffee, toasted coffee, chlorogenic acid (CGA), and caffeic acid (CA) against SW480 and SW620 with high IC50 values. Our evidence supports the antiproliferative activity of green coffee (IC50 2555 µg/mL), toasted coffee (IC50 2226 µg/mL), CGA (IC50 598.3 µg/mL), and CA (IC50 161.4 µg/mL) on SW480 (Villota et al., 2021). Although coffee extracts showed higher IC50 values, the extraction process was based on chemical methods. In this study, polysaccharides reached a lower IC50 concentration with evidence of a polysaccharide chain.

Therefore, compared to other studies, the C. gileadensis polysaccharides extract showed potent pharmaceutical activities against the SW480 cell line. Although the antiproliferative effects of C. gileadensis polysaccharides on SW620 showed higher IC50 32.02 µg/mL, this would be considered a potent therapeutic agent. In addition , the antiproliferative dose used, which is the IC50, showed some resistance toward the cellular apoptosis pathway. The only significant results toward the apoptosis pathway were determined when the cells were treated with 2× and 4× the IC50 over 24 h (p 0.01). Thus, it would be explained that the SW620 is a metastatic-derived cell line (Hewitt et al., 2000). The antiproliferative activities on the SW620 cell line derived from Chitooligosaccharide and gallic acid showed an IC50 with 62.5 µg/mL (Saetang et al., 2023). Herrera-Calderon et al. examined the effect of hydroethanolic extracts derived from Dodonaea viscosa , which showed antiproliferative effects on SW620 with 87.7 µg/ mL(Herrera-Calderon et al., 2023).

Regarding antiproliferative activities of C. gileadensis , several studies revealed antiproliferative effects of C. gileadensis chemical extracts, for example, the effect of C. gileadensis ethanolic extract on two cell lines, namely, mouse lymphoma cell line (BS-24-1) an Epstein-Barr virus-transformed human B lymphocyte (MoFir) (Amiel et al., 2012). Another study used chemical extraction of C. gileadensis to produce sesquiterpenoids using petroleum ether to have antiproliferative effects on prostate cancer cell lines with IC50 31 µM (Shen et al., 2007). Another study showed antiproliferative activities of C. gileadensis ethanolic extracts on HaCaT keratinocytes, human dermal fibroblasts (HDF), and human dermoid carcinoma A431 (Wineman et al., 2015). Several extraction techniques

were used to evaluate the antiproliferative activities of C. gileadensis; however, this is the first study that revealed the antiproliferative effects of polysaccharides derived from C. gileadensis on CRC cell lines.

CONCLUSION AND RECOMMENDATION

C. gileadensis polysaccharide extracts have potent antiproliferative activity, which induces apoptosis on CRC cell lines. Further separation and characterisation methods should be applied to determine the active compound causing cellular death.

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DECLARATIONS

Conflict of interest: The authors have no relevant financial or non-financial interests to disclose. The authors declare no conflict of interest.

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