Carrageenan: Future Potential Ingredient of Lubricant and Feminine Hygiene Product with Possible Protection Effects against HPV Infection

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Abstract
The discovery that human papillomavirus (HPV) infection is the primary cause of cervical cancer has opened up new avenues for prevention. Carrageenans are attractive candidates for developing potential HPV prevention due to their actions against a wide range of viruses, mainly through the blocking of the viral attachment stage. In addition, they are characterised by low production costs, abundant availability, biodegradability, biocompatibility, and non-toxicity. This review presents an overview of in vitro and in vivo studies of carrageenan antiviral properties, availability, and future liquid-sexual material. Based on the results of previous studies, both in vitro and in vivo carrageenan has the potential to be applied as a lubricant and feminine cleanser because it can reduce HPV infection, is non-toxic, and non-allergenic.

Keywords
Cervical cancer, human papilloma viruses (HPV), lubricant, feminine cleanser biomedical products

1 Introduction
Cervical cancer is cancer that grows in the cervix cells and occurs when healthy cells undergo changes or mutations. This mutation causes these cells to grow abnormally and uncontrollably, forming cancer cells.1 Cervical cancer is one of the most common malignant tumour in women globally, but primarily occurs in underdeveloped or developing countries. In 2020, an estimated 604,127 (3.1 %) women were diagnosed with cervical cancer and about 341,831 (3.4 %) died due to the disease.2,3 Almost all cervical cancers are mainly caused by high-risk human papillomavirus (HPV) infection. However, the other cofactors of cervical cancer are sexually transmittable infections (HIV and Chlamydia trachomatis), smoking, long-term use of oral contraceptives, high age, parity, and low socioeconomic status.4–6

The discovery that human papillomavirus (HPV) infection is the primary cause of cervical cancer has opened up new avenues for prevention, including prevention of infection, improved screening methods, and HPV vaccine.2,6 Cervical cancer treatment is based on the characteristics and stage of cancer. The main treatments for cervical cancer are surgery, gene therapy, radiotherapy, chemotherapy, and immunomodulatory therapy.9,10

Gene therapy is understood as the ability of genetic improvement through the correction of altered (mutated) genes or site-specific modifications that target therapeutic treatment.12 Radiotherapy is a cancer treatment that uses high doses of radiation to kill cancer cells and shrink tumours.11 Chemotherapy is the use of chemicals to treat disease. Immunotherapy is a type of treatment to encourage the work of the immune system or the immune system to be more effective in fighting cancer.13 The mechanism of action of each therapy is different for inhibiting cell division and proliferation of rapidly growing cells.14 Chemotherapy is generally and effectively used in treatment of cancer, destroying not only malignant cells but also normal cells with high-proliferating potential.15 The most common side effects of chemotherapy are nausea and vomiting, fatigue, loss of appetite, hair loss, dry mouth, and constipation.16,17 Thus, the discovery of new anticancer drugs with high activity and low side effects is an imperative. According to Nurgali,18 the combination of natural bioactive compounds with traditional chemotherapeutic drugs can enhance anticancer efficacy and reduce side effects of chemotherapy.

Marine organisms provide a rich source of the novel anticancer drug and an alternative source to meet the demand for effective and cut-price drugs.19,20 Numerous bioactive compounds from marine organisms show various therapeutic effects and pharmacological properties, such as antioxidant, antitumour, anticancer, and antiviral.21–23 More than 36,000 marine-derived compounds have been reported, and over 1,500 compounds more are delineated each year.24 There are many marine-derived compounds in preclinical, clinical pipeline, and some marine-derived compounds have been put on the market.25 In November 2019, six marine-derived drugs for cancer were approved in the clinical marine pharmaceutical pipeline including cytarabine, eribulin mesylate, brentuximab vedotin, trabectedin, plitidepsin, and polatuzumab vedotin.26

Seaweeds, also known as marine microalgae, are widely distributed in the ocean including the intertidal, tidal and subtidal regions, respectively.27 They are divided into three major groups; brown algae or phaeophyceae, green algae or chlorophyta, and red algae or Rhodophyta.28 Seaweeds contain medicinally potent compounds, including flavo-
Carrageenans are a group of linear sulphated polysaccharides that are made up of repeating structure of galactose units and 3,6-anhydro-galactose (3,6-AG). The units are joined by α-1,3 and β-1,4-glycosidic linkage. Several studies have shown that sulphated seaweed polysaccharides, including carrageenan, have promising effects not only against different cancer cell types in vitro and in vivo, but also against different types of viruses. Due to its biological activity, the use of carrageenan as a naturally occurring polysaccharide, has been increasing widely for human applications, thus creating a strong position in the biomedical field. The present review is focused on the application of carrageenan biomedical products to prevent and treat cervical cancer.

### 2 Carrageenan from red seaweeds

Carrageenans are mostly extracted from red seaweeds, particularly from Chondrus crispus, Kappaphycus alvarezi, and Eucheuma denticulatum. Other genera, including Gigartina, Iridaea, hypnea, Acanthophora, Porphyra umbilicalis, Meristotheca, Cystocloniaceae, and Sarconema are also used as sources of carrageenan. Species of Eucheuma and Hypnea predominantly produce κ-Carrageenan and ι-Carrageenan, while the genera of Gigartina and Chondrus are the source of λ-Carrageenan, and the genus of Cystocloniaceae family mostly produce iota-Carrageenan. In recent years, some processes for the extraction of carrageenan from various red seaweed species have been reported. These processes include enzyme-assisted extraction (EAE), physical processes, chemical extraction (alkaline treatment), and microwave-assisted extraction (MAE). Carrageenans are a group of linear sulphated polysaccharides that are made up of repeating structure of galactose units and 3,6-anhydro-galactose (3,6-AG). The units are joined by α-1,3 and β-1,4-glycosidic linkage. Based on the family, carrageenan is classified mainly into four types, including kappa-carrageenan (κ-carrageenan), beta-carrageenan (β-carrageenan), lambda-carrageenan (λ-carrageenan), and omega-carrageenan (ω-carrageenan). The kappa (κ) family contains a subclass such as kappa (κ), iota (ι), mu (μ), nu (ν), and omicron (ο) carrageenans. The beta (β) family comprises beta (β) and alfa (α)-carrageenans and their biological precursors gamma (γ) and delta (δ)-carrageenan. The lambda (λ) family contains a subclass including lambda (λ), xi (ξ), pi (π), and theta (θ) carrageenan, while the omega (ω) family contains a subclass including omega (ω) and its biological precursors psi (ψ) carrageenan.

<table>
<thead>
<tr>
<th>Carrageenan</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>Carrageenan</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
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<tr>
<td>κ</td>
<td>H</td>
<td>SO₃⁻</td>
<td>H</td>
<td>H</td>
<td>μ</td>
<td>H</td>
<td>SO₃⁻</td>
<td>H</td>
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<td>ι</td>
<td>H</td>
<td>SO₃⁻</td>
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<td>SO₃⁻</td>
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<td>α</td>
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<td>H</td>
<td>ξ</td>
<td>SO₃⁻</td>
<td>H</td>
<td>H</td>
<td>SO₃⁻</td>
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<td>ω</td>
<td>H</td>
<td>H</td>
<td>SO₃⁻</td>
<td>H</td>
<td>π</td>
<td>SO₃⁻</td>
<td>Pyruvate</td>
<td>SO₃⁻</td>
<td>H</td>
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<tr>
<td>θ</td>
<td>SO₃⁻</td>
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<td>H</td>
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<td>SO₃⁻</td>
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</table>

**Fig. 1** – Structures of carrageenans
The solubility of carrageenan is influenced by various factors, such as temperature, pH, the presence of other solutes, the type of carrageenan (sulphate groups), and their associated cations such as K+ and Ca2+. All carrageenans are soluble in hot water. However, λ-carrageenan and sodium salt of κ- and ι-carrageenans are soluble in cold water. The viscosity of the solutions is increased by λ-carrageenan, which does not gel. At low concentrations, κ- and ι-carrageenans produce gels when cooled, depending on the additional cation (calcium or potassium) at low concentration 0.5%. κ-carrageenan gels are weaker in the presence of Ca2+ than K+. In the presence of Ca2+, κ-carrageenan gels are stronger yet brittle, and they tend to show syneresis (separation of liquid from its gel). In the presence of Ca2+, λ-carrageenan forms flexible gels with little syneresis.

### Table 1 – Physical characteristics of commercial carrageenans

<table>
<thead>
<tr>
<th>CG types</th>
<th>Iota (ι)</th>
<th>Kappa (κ)</th>
<th>Lambda (λ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gelling/°C – effect cations – gel texture</td>
<td>strongest gel with Ca2+</td>
<td>strongest gel with K+</td>
<td>non-gelling –</td>
</tr>
<tr>
<td>solubility / 80°C</td>
<td>soluble</td>
<td>soluble</td>
<td>soluble</td>
</tr>
<tr>
<td>melting/°C</td>
<td>50–80</td>
<td>40–75</td>
<td>–</td>
</tr>
<tr>
<td>pH stability</td>
<td>4–10</td>
<td>4–10</td>
<td>4–10</td>
</tr>
<tr>
<td>sources:</td>
<td>49,55</td>
<td></td>
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</tr>
</tbody>
</table>

3 Mechanism of antiviral action

Carrageenan has high density of anionic groups from the presence of the sulphate residues in their molecular structure. Ionic interactions between anionic groups in the polysaccharide and basic amino acids of the glycoprotein form the virus-cell complex, while non-ionic interactions rely on hydrophobic amino acids interspersed between the basic ones in the glycoprotein-binding zone. Carrageenans are selective inhibitors of several enveloped viruses (HIV-1, HSV-1, IFV-A, IFV-B, RSV-A, RSV-B, and PIVV-2), and non-enveloped viruses (HAV and HPV), and act predominantly by inhibiting viral binding, inhibiting viral internalisation and un-coating, or inhibiting viral transcription and replication. The mechanism of viral inhibition of carrageenan is shown in Fig. 2. Viral infections require binding to the surface of the host cell. Various cell surface components may play a role in viral attachment, and among them, glycans displayed on proteins or surface lipids interact with a large number of viruses. In enveloped viruses, this blocking capacity has been reported, possibly due to the ability of carrageenan to bind to viral envelope proteins, such as herpes simplex glycoproteins. After attachment, carrageenans can block viruses from nucleocapsid internalisation into the cytoplasm, and disable them to uncoat from the endosome. In addition, the presence of carrageenan can inhibit the reverse transcriptase activity of virus.

Carrageenan has been found to be an exceptionally powerful inhibitor of HPV infection. The inhibitory effect of carrageenan on HPV-PsV infection of various cell types was validated by in vitro clinical trials (HeLa cells, HaCaT cells, sperm cells, HSPG-deficient PCSA-745 cells, and 293TT cells). The most widely accepted explanation for this effect is that carrageenan binds directly to the capsid. Based on reported studies, thirteen women were enrolled, and thirty samples taken after various time intervals were evaluated. Carrageenans were detected in 87% of CVL samples, with levels decreasing with time as a result of intercourse. PsV16 inhibition was detected in 93% of CVL samples, with a median inhibition of 97.5%. PsV16 inhibition reduced over time but remained high, with median inhibition of 98.1%, 97.4%, and 83.4% after 1, 4, and 8–12 h, respectively. Higher concentrations of carrageenans were found to be related to higher levels of PsV16 inhibition. Using a carrageenan-based lubrication gel can help women avoid genital HPV infections. The lubricating gel can, apart from women, also be used by men for application on condoms or penis.

Carrageenan and carrageenan oligosaccharides possess well-known antiviral properties, and are reported to be effective mainly against papillomavirus (HPV) infection. The inhibitory effect of carrageenan on HPV-PsV infection of various cell types was validated by in vitro clinical trials (HeLa cells, HaCaT cells, sperm cells, HSPG-deficient PCSA-745 cells, and 293TT cells). The most widely accepted explanation for this effect is that carrageenan binds directly to the capsid. Based on reported studies, thirteen women were enrolled, and thirty samples taken after various time intervals were evaluated. Carrageenans were detected in 87% of CVL samples, with levels decreasing with time as a result of intercourse. PsV16 inhibition was detected in 93% of CVL samples, with a median inhibition of 97.5%. PsV16 inhibition reduced over time but remained high, with median inhibition of 98.1%, 97.4%, and 83.4% after 1, 4, and 8–12 h, respectively. Higher concentrations of carrageenans were found to be related to higher levels of PsV16 inhibition. Using a carrageenan-based lubrication gel can help women avoid genital HPV infections. The lubricating gel can, apart from women, also be used by men for application on condoms or penis.

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products are effective as topical microbicides against genital HPV.

According to Derby et al., griffithsin (GRFT) combined with carrageenan (CG) has strong activity in vitro and in vivo against herpes simplex virus-2 (HSV-2) and human papillomavirus (HPV). They have reported that GRFT/CG in a freeze-dried fast dissolving insert (FDI) formulation for on-demand use protects rhesus macaques from a high dose vaginal SHIV SF162P3 challenge 4 h after FDI insertion. Furthermore, the GRFT/CG FDI also protects mice vaginally against HSV-2 and HPV pseudovirus. The GRFT/CG warrants clinical development. Prasedya et al. concluded that loss of normal cell-cycle control, that underlies tumor growth. Recently there is an increasing interest in potential anticancer agents that affect cell cycle in cancer cells. Thus, in this study we investigated the effects of carrageenan on the tumor cell cycle. Methods: Using human cervical carcinoma cells (HeLa) has reported that κ-carrageenan and λ-carrageenan have no significant effect on human umbilical vein endothelial cells (HUVEC). In contrast, both forms of carrageenan were cytotoxic towards HeLa cells (cancer cells).

Carrageenan inhibits not only papillomavirus (HPV) infection but also cofactor viruses of cervical cancer, such as human immunodeficiency virus (HIV) and Chlamydia trachomatis infections. Iota-carrageenan could be a promising agent to reduce the transmission of ocular chlamydial infection, and opens perspectives to develop prophylactic approaches to block C. trachomatis entry into the host cell. In vitro and in vivo antiviral activities of carrageenan are shown in Tables 2 and 3.

4 Future prospect of application

Why carrageenan has potential? Carrageenan has many applications in food (dessert mousse, canned food, ice cream, bakery fillings, instant desserts, and salad dressings), and medicine (stabilisers and suspension agents in some other drugs, medicinal creams, and lotions). According to Grand View Research Data in 2021, the global carrageenan market was worth USD 780.5 million in 2020, and it is predicted to increase at a CAGR of 6.0 percent from 2020 to 2028. Carrageenan’s mouthfeel properties to replicate fatty feeling are predicted to enhance market growth during the projected period, resulting in increased product penetration in dairy and processed meat products.

Based on its biological activities, carrageenans have been extensively investigated for their bioactivities such as anticoagulant, anticancer, antiviral, cholesterol-lowering effects, immunomodulatory activity, and antioxidant. Carrageenan possesses promising activity both in vitro and in vivo, showing promising potential to be developed as HPV prevention agents. In addition, previous studies back up the theory that CG aids in the natural clearance of genital HPV infection in women who have a positive HPV-DNA test, acceptable in this population of HIV-infected women, and is nontoxic. According to the theory that CG aids in the natural clearance of genital HPV infection in women who have a positive HPV-DNA test, acceptable in this population of HIV-infected women, and is nontoxic. According to the theory that CG aids in the natural clearance of genital HPV infection in women who have a positive HPV-DNA test, acceptable in this population of HIV-infected women, and is nontoxic.

Table 2 – In vitro evaluation of carrageenan to prevent HPV

<table>
<thead>
<tr>
<th>CG Type</th>
<th>Virus Type</th>
<th>Cell Line</th>
<th>IC50</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ/ι-CG</td>
<td>HPV16, 18, and 45 PsVs</td>
<td>HeLa cells</td>
<td>1–20 ng ml⁻¹</td>
<td>72</td>
</tr>
<tr>
<td>ι-CG</td>
<td>BVPI</td>
<td>C127 cells</td>
<td>1–10 µg ml⁻¹</td>
<td>62</td>
</tr>
<tr>
<td>CG (PC-1005)</td>
<td>HPV16, HIV</td>
<td>HeLa cells</td>
<td>&gt;100 nM</td>
<td>73</td>
</tr>
<tr>
<td>CG</td>
<td>MT-4 cells</td>
<td>100 µg ml⁻¹</td>
<td>74</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 – In vivo evaluation of carrageenan to prevent HPV

<table>
<thead>
<tr>
<th>CG Type</th>
<th>Virus Type</th>
<th>Experimental System</th>
<th>Effects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>HPVs</td>
<td>380 participants gbMSM receive treatment CG</td>
<td>Inhibition of infection.</td>
<td>77</td>
</tr>
<tr>
<td>CG-based lubricant (Divine 9)</td>
<td>HPV</td>
<td>During each act of vaginal intercourse, 280 women utilised gel plus condoms.</td>
<td>Infection with HPV is less common.</td>
<td>66</td>
</tr>
<tr>
<td>λ/ι-CG (carraguard)</td>
<td>HPVs</td>
<td>During each act of vaginal intercourse, 1718 women utilised gel plus condoms.</td>
<td>Infection with high-risk HPV is less common.</td>
<td>78</td>
</tr>
<tr>
<td>CG (0.02 %) + Propionibacterium extract (CGP)</td>
<td>HPVs</td>
<td>The study involved 40 healthy, sexually active women between the ages of 18 and 45 who had genital HPV infection. CG-CGP was given to each subject.</td>
<td>CG helps to speed up the usual clearance of genital HPV infection.</td>
<td>79</td>
</tr>
<tr>
<td>CG (carraguard)</td>
<td>HIV</td>
<td>60 women enrolled with a median age of 34 years; 25 % were sexually active. CG (carraguard) was given to each subject.</td>
<td>Higher perceived need for protection among HIV-infected women.</td>
<td>80</td>
</tr>
</tbody>
</table>
uct. Based on its physicochemical properties, biological activities, and abundant availability, carrageenan has the potential as a raw material for medical products such as lubricant gel and feminine cleanser Fig. 3.

**Lubricant gel**

Most people are safe to use commercial lubricants. However, some lubricant products present risks such as allergic reactions, skin irritation, yeast infections, interfering with fertility, drying up rapidly, and requiring frequent reapplication. Some lubricants may affect function of sperm, decreasing the opportunity of a woman to conceive. Those attempting to conceive should use a sperm-friendly lubricant. Sexual couples wishing to use lubricants and prevent pregnancy can consider using spermicides combined with other contraceptives. A study has shown that the use of lubricants during procreative intercourse does not decrease the chances of conceiving.^

**Feminine cleanser**

Intimate hygiene products are used by women on a regular basis as part of their cleaning routine. Minimising the onset or worsening of an inflammatory illness while maintaining high levels of infection protection, daily feminine hygiene should be approached with care in selecting an appropriate solution. Feminine cleanser has no negative effects on sperm motility.^

Carrageenan is a group of linear sulphated polysaccharides that are extracted from red seaweed. They are selective inhibitors of several enveloped viruses and non-enveloped viruses including HPV, HIV, and Chlamydia trachomatis. The virucidal properties of carrageenan may be related to the virus’s inability to complete the infection process, because the viral envelope sites essential for virus attachment to host cells are occupied by the sulphated polysaccharide. Some studies have reported that both in vitro and in vivo carrageenans have the potential to be applied as a lubricant and feminine cleanser products, because they not only protect against genital HPV and reduce infection of HPV and cofactor viruses of cervical cancer, but are also non-toxic for human cells.

**5 Conclusions**

Carrageenans are a group of linear sulphated polysaccharides that are extracted from red seaweed. They are selective inhibitors of several enveloped viruses and non-enveloped viruses including HPV, HIV, and Chlamydia trachomatis. The virucidal properties of carrageenan may be related to the virus’s inability to complete the infection process, because the viral envelope sites essential for virus attachment to host cells are occupied by the sulphated polysaccharide. Some studies have reported that both in vitro and in vivo carrageenans have the potential to be applied as a lubricant and feminine cleanser products, because they not only protect against genital HPV and reduce infection of HPV and cofactor viruses of cervical cancer, but are also non-toxic for human cells.

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24. Advances in cervical cancer, different treatments

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27. Advances in cervical cancer, different treatments

28. Advances in cervical cancer, different treatments

29. Advances in cervical cancer, different treatments

30. Advances in cervical cancer, different treatments


SAŽETAK
Karagenan: budući potencijalni sastojak lubrikanta i proizvoda za žensku higijenu s mogućim zaštitnim učinkom protiv HPV infekcija
Jumardi Jumardi, Rugaiyah A. Arfah i Nur Umriani Permatasari


Ključne riječi
Rak grlića maternice, humani papiloma virusi (HPV), lubrikant, biomedicinski proizvodi za higijenu žena

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Pregledni rad