



Analysis of Flavonoid Bioactives in Combating HPV Infection: Potential for the Development of Cervical Cancer Therapy

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Abstract

Introduction: Human Papilloma Virus (HPV) infection is the main cause of cervical cancer and ranks fourth most common cancer in women worldwide. The high incidence rate and current limitations of treatment are the basis for studying the potential of flavonoid compounds which are natural resources of Indonesia as an alternative anti-HPV therapy.

Method: The method used is a narrative literature review with literature collection carried out through the PubMed, ScienceDirect, and Google Scholar databases. The selected articles were those published in the last ten years (2014-2024) and are available in abstract and full text.

Results: Flavonoids have significant potential as anti-HPV agents with mechanisms involving apoptosis induction and inhibition of oncoprotein pathways, such as E6 and c-Myc, in cervical cancer cells. Several natural sources such as banana peel with a content of 20.76 mg qe/g, seeds of the fruit of the boils with a content of 423.60 mg qe/g, and leaves of the matoa with a content of 15.884 mg qe/g are proven to be rich in flavonoids and offer opportunities for the development of HPV infection treatment therapy.

Conclusion: Flavonoids have good potential as anti-HPV in preventing cervical cancer with different mechanisms according to the type of compound. Flavonoid content can be found in banana peel, seeds of the fruit of the boils, red onion waste, and matoa.

Keywords: Anti-HPV, Flavonoids, HPV, Natural ingredients, Plant extracts

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INTRODUCTION

Human papillomavirus (HPV) infection is one of the most prevalent sexually transmitted infections worldwide and ranks first in the United States (Stelzle et al., 2021). HPV is a double-stranded DNA virus belonging to the family Papillomaviridae. This virus forms a large and diverse group, with new HPV variants continually being identified over time. Based on their risk, HPV types are classified into low-risk and high-risk HPV. When high-risk HPV infects cervical cells, the virus disrupts the way cells replicate and divide, causing infected cells to proliferate uncontrollably. This subsequently becomes the precursor to cervical cancer (Bzhalava, Eklund and Dillner, 2015).

Cervical cancer remains a health problem for which no definitive solution for complete management has yet been established. Cervical cancer ranks fourth among the most common cancers in women worldwide, with approximately 660,000 new cases and about 350,000 deaths in 2022. In Indonesia, this cancer once ranked second after breast cancer, with 36,633 cases and 21,003 deaths (GLOBOCAN, 2020). What distinguishes cervical cancer from other cancers is its clearly established cause, namely HPV infection.

Although preventive measures against cervical cancer, such as HPV vaccination and early detection (visual inspection with acetic acid [VIA]), have been implemented, the incidence of cervical cancer remains relatively high; therefore, therapeutic treatment continues to be administered to date. In addition, current treatments do not completely cure the disease and have side effects that may lead to worsening at certain times (Palagudi et al., 2024). Based on this,

researchers are motivated to seek appropriate alternative solutions to address it, one of which is by examining and utilizing available natural resources.

Indonesia is an agrarian country with abundant natural resources. This represents a potential for utilizing resources available in the surrounding environment. Potential resources that can be used include secondary metabolite compounds from plants, such as flavonoids. Flavonoids are a group of natural substances with diverse phenolic structures and can be found in fruits and vegetables, grains, bark, roots, stems, and other plant parts (Panche, Diwan and Chandra, 2016). The variation of flavonoids among plants and Indonesia’s biodiversity strongly support the development of products derived from available natural materials. Currently, the use of natural materials for health maintenance is still urgently needed, especially given the high costs of treatment and medicines (Limbu, U.N. Bao, A.P Azi, 2024).

Based on the above description, more in-depth knowledge and study are needed regarding the role of flavonoids as anti-HPV agents and their potential effects in humans. The research findings presented in this literature review can serve as a collective review of the potential of flavonoid compounds from natural sources as anti-HPV agents in preventing the development of cervical cancer in Indonesia.

METHOD

The method employed in this literature review was a narrative literature review. Literature data were collected from three different databases: PubMed, ScienceDirect, and Google Scholar, including articles written in either Indonesian or English. The keywords used were flavonoid, HPV, anti-HPV, plant extract, and natural product. The article search was limited to recent articles or journals; therefore, the publication year was restricted to the last 10 years, namely 2014–2024. The articles used were original articles. The selected articles were those for which abstracts and full texts were available. Article selection was based on the objectives of the review, namely to determine the potential of flavonoids as anti-HPV agents and to identify which natural products can serve as sources of flavonoids.

RESULTS

HPV is a double-stranded DNA virus belonging to the family Papillomaviridae and infects the skin and mucosal epithelium. To date, more than 200 HPV types that infect the human body have been identified, with approximately 40 types transmitted through sexual contact (Graham, 2017). These HPV types are generally classified into two major groups based on risk: low-risk HPV and high-risk HPV. The diseases caused also differ depending on the site of HPV infection. Further details are presented in Table 1.

Table 1. Sites of HPV Infection (Graham, 2017)

Infection Site	HPV-Associated Disease	HPV Types
Skin	Warts	1,2,3,4,7,10,26,27,28,29,41, 48,50,57,60,63,65,75,76,77, 88,95
	<i>Epidermodysplasia verruciformis</i>	5,8,9,12,14,15,17,19,20,21,22,23,24, 25,36,37,38,46,47,49, 75,76,80,92,93, 96
Oropharynx, larynx, and oral cavity	Head and neck cancer	16
Oral cavity	<i>Focal epithelial hyperplasia</i>	13,32
Larynx	<i>Laryngeal papillomatosis</i>	6,11
Anogenital are	Genital warts	6,11
	<i>Intraepithelial neoplasia</i>	40,42,43,44,53,54,61,72,73, 81

Infection Site	HPV-Associated Disease	HPV Types
	<i>Intraepithelial neoplasia dan kanker serviks</i>	16,18,26,31,33,35,39,45,51, 52,56,53,58,66,68,73,82

Flavonoids are secondary metabolites secreted by plants. Flavonoids provide various therapeutic applications in humans, including cardioprotective, antidiabetic, and antiviral activities, as well as protection for plants in response to stress. Vegetables, flowers, and grains are rich sources of flavonoids (Roy et al., 2022). Flavonoid compounds are synthesized from phenylalanine and malonyl-CoA, and more than 8,000 flavonoids have been identified in plants (Alseekh et al., 2020). Flavonoids have various bioactive effects, one of which is antiviral. These compounds contain 15 carbon atoms arranged in a C6-C3-C6 configuration (Wang et al., 2016).

Flavonoid types are divided into several classes based on the C carbon ring, to which the B carbon ring is attached, as well as the degree of unsaturation and oxidation of the C ring. Flavonoids consist of flavones, flavonols, flavanones, flavanonols, flavanols, catechins, anthocyanins, and chalcones (Panche, Diwan and Chandra, 2016). Further details can be seen in the flavonoid classification in Table 2.

Table 2. Classification and Content of Flavonoids and Their Sources (Panche, Diwan and Chandra, 2016)

Class	Content
<i>Anthocyanin</i>	<i>Cyanidin, malvidin, delphinidin, dan peonidin.</i>
<i>Chalcones</i>	<i>Phloretin, arbutin, phloridzin, dan chalconaringenin.</i>
<i>Flavanones</i>	<i>Apigenin, tangeretin, baicalein, dan rhoifolin.</i>
<i>Flavonols</i>	<i>Quercetin, myricetin, rutin, morin, dan kaempferol.</i>
<i>Isoflavonoid</i>	<i>Genistin, genistein, daidzein, glycitein, dan daidzin.</i>

Flavonoids are products of plant metabolism well known for their versatile biological benefits. These compounds have relatively strong antiviral activity against many viruses. Testing of these materials showed more than 90% antiviral activity without affecting cell viability (Lal et al., 2021). To determine the effect of flavonoids as anti-HPV based on previous studies, see Table 3.

Table 3. Studies on Flavonoids as Anti-HPV

Title	Method	Year	Hasil
Flavonol and imidazole derivatives block HPV16 E6 activities and reactivate apoptotic pathways in HPV+ cells	In vitro experimental study	2016	Both myricetin and spinacine sensitized HPV-positive cervical cancer cells and induced apoptosis mediated by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Yuan et al., 2016).
RPS12 increases the invasiveness in cervical cancer activated by c-Myc and inhibited by the dietary flavonoids luteolin and quercetin	In vitro experimental study	2015	Expression of c-Myc and AKT/mTOR in ribosomal protein 12 in A431-III cells was significantly reduced by luteolin and quercetin treatment (Lin et al., 2015).
Chemopreventive Role of Apigenin against the Synergistic Carcinogenesis of Human Papillomavirus and 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	In vitro experimental study	2020	The possible preventive mechanism associated with apigenin is G1-phase cell cycle arrest by downregulating mutp53, CDK4, cyclin D1, and p-Rb, and by blocking NNK metabolic activation catalyzed by P450 (Yin et al., 2020).

Title	Method	Year	Hasil
Linarin as an Anti-Cancer Therapeutic: In-Silico and Machine Learning Approach for HPV	In silico study (computer simulation)	2023	Linarin can be easily extracted from angiosperms and used as an herbal product to inhibit HPV E7 oncoprotein (Bhardwaj, Ananya and Das, 2023).
Development and Characterization of Quercetin-Loaded Delivery Systems for Increasing Its Bioavailability in Cervical Cancer Cells	In vitro experimental study	2023	Quercetin is a natural flavonoid with high anticancer activity, especially for HPV-related cancers such as cervical cancer (Ferreira et al., 2023).
Quercetin induces G2 phase arrest and apoptosis with the activation of p53 in an E6 expression-independent manner in HPV-positive human cervical cancer-derived cells	In vitro experimental study	2019	Quercetin can reactivate p53 and induce G2-phase cell cycle arrest and apoptosis in HPV-positive human cervical cancer-derived cells by inhibiting E6/E6AP interaction (Clemente-Soto et al., 2019).
Antitumor Effect of Chalcone Derivatives against Human Prostate (LNCaP and PC-3), Cervix HPV-Positive (HeLa) and Lymphocyte (Jurkat) Cell Lines and Their Effect on Macrophage Functions	In vitro experimental study	2023	For the HeLa cell line (HPV-positive cervical cells), the compound showed low-to-moderate inhibition of metabolic activity at 20 μ M (Lncap et al., 2023).
Anti-Cancerous Effect of Rutin Against HPV-C33A Cervical Cancer Cells via G0/G1 Cell Cycle Arrest and Apoptotic Induction.	In vitro experimental study	2020	Rutin triggers apoptosis by inducing a decrease in mitochondrial membrane potential and activation of caspase-3. Cell cycle analysis further confirmed rutin's efficacy by showing cell cycle arrest at G0/G1 phase (Khan F, Pandey P, Upadhyay TK, 2020).
TLR4-MyD88-TRAF6-TAK1 Complex-Mediated NF- κ B Activation Contribute to the Anti-Inflammatory Effect of V8 in LPS-Induced Human Cervical Cancer SiHa Cells.	In vitro experimental study	2018	The synthetic compound 7-4-[Bis-(2-hydroxyethyl)-amino]-butoxy-5-hydroxy-8-methoxy-2-phenylchromen-4-one (V8) is a new flavonoid derivative. V8 decreases expression of NF- κ B target genes, such as COX-2, iNOS, IL-6, IL-8, CCL-2, and TNF- α in SiHa cells (He A, Ji R, Shao J, He C, Jin M, 2016).
Oxidative Stress Triggered by Apigenin Induces Apoptosis in a Comprehensive Panel of Human Cervical Cancer-Derived Cell Lines	In vitro experimental study	2017	Apigenin has strong and selective antitumor effects on cervical cancer cells immortalized by HPV 16, HPV 18, and both HPV 16 and 18 (Souza et al., 2017).

DISCUSSION

Pathophysiology of Cervical Cancer

The development of cervical cancer involves multiple pathways, one of which begins with human papillomavirus (HPV) infection. HPV types 16 and 18 are the high-risk HPV types most frequently triggering cervical cancer, unlike other types such as HPV 6 and 11, which are less virulent and cause warts (Akbar et al., 2019). Initially, the virus enters the basal membrane of the vaginal mucosa by exploiting abrasions or lesions in epithelial cells. HPV then binds to heparan sulfate proteoglycan (HSP) receptors, a process assisted by several proteins such as syndecan-1 and laminin-332. Subsequently, the HPV capsid undergoes changes assisted by cyclophilin-B,

and capsid cleavage is mediated by furin. The virus then enters cervical cells through endocytosis (Cagno et al., 2019). The virus then enters stem cells in the cervix and controls their proliferation. The process by which proliferation is altered remains widely studied because many pathways may enable it. Several pathways act through overexpression of proteins in neoplasms, such as PI3K (phosphatidylinositol 3-kinase), EGF-R, β -catenin, Erk, antiapoptotic proteins such as the Bcl-2 subfamily, and the DEG pathway involving nine genes (Wu et al., 2019; Pal and Kundu, 2020). In addition to these pathways, increased expression of HPV oncoproteins E6 and E7 is the main oncogenic trigger for cellular transformation in cervical cancer. E6 induces degradation of important cell cycle regulators such as proteins containing p53 and PDZ domains, whereas E7 promotes pRb inactivation. E6 and E7 contribute to the “hallmarks of cancer,” namely sustaining proliferative signaling, evading growth suppressors, resisting apoptosis, enabling replicative immortality, sustaining angiogenesis, and tissue invasion and metastasis through various molecular processes (Manzo-Merino et al., 2014). One such pathway is shown in Figure 1.

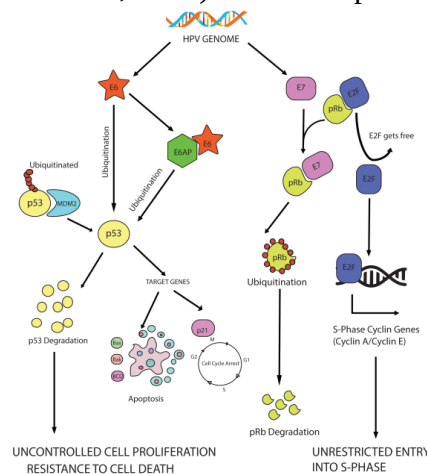


Figure 1. Role of E6 and E7 in Manipulating Cell Proliferation (Manzo-Merino et al., 2014).

Mechanisms of Flavonoids as Anti-HPV

Based on Table 3, previous studies were grouped according to the mechanism of flavonoid-derived compounds against HPV infection. From our review of the table, flavonoid derivatives with anti-HPV properties are quercetin, myricetin, linarin, apigenin, and luteolin [6,7,8,9].

As shown in Figure 2, concentrations of 0–125 μ M of myricetin (a flavonoid derivative) and spinacine (an imidazole derivative) added to SiHa cells (derived from cervical carcinoma) with the addition of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) reduced SiHa cell viability by 30–40% at a dose of 125 μ M. Myricetin and spinacine make cells more sensitive to intrinsic apoptosis triggered by chemotherapeutic drugs, such as doxorubicin and cisplatin. This may be clinically relevant because cervical cancer tends to be relatively resistant to chemotherapy, such as cisplatin. Myricetin and spinacine can block E6 binding to several apoptotic proteins, including caspase 8 and E6AP/p53, thereby reactivating apoptotic pathways disrupted by E6 and making HPV+ cells sensitive to intrinsic and extrinsic apoptosis inducers (Yuan et al., 2016).

In studies with cervical cancer cells, ribosomal protein 12 was detected in cervical cancer tissue but not in normal tissue. This indicates that this protein gene can be used as an early molecular diagnostic marker in human cervical cancer screening and as a potential therapeutic target. Ribosomal protein 12 is affected by increased c-myc protein and can be inhibited by administration of flavonoid derivatives, namely luteolin and quercetin. The result was a significant decrease in expression of phosphorylated AKT, mTOR, and S6K after administration of luteolin at 20 μ M and quercetin at 40 μ M. In addition, c-myc protein was also significantly reduced by this intervention (Lin et al., 2015).

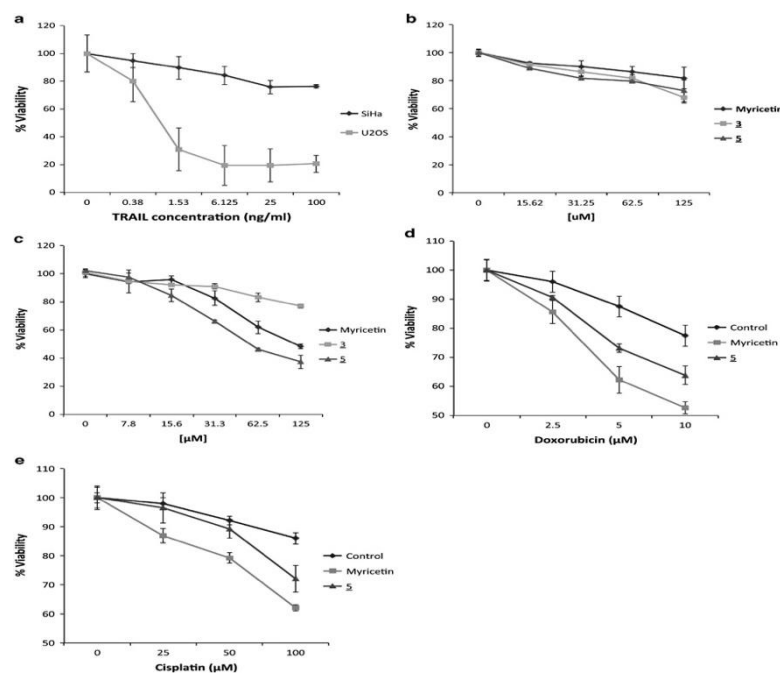


Figure 2. SiHa Cell Viability After Intervention with TRAIL, Myricetin, Doxorubicin, Cisplatin (Yuan et al., 2016).

A marked increase in mutp53 expression in experimental cells was observed after exposure to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, indicating that this compound and HPV18-E6E7 together promote cancer development by reducing the tumor suppressor effect of p53. Mutation of the p53 gene induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone subsequently caused upregulation of cyclin D1, CDK4, and p-Rb and resulted in a significant increase in the percentage of cells entering S phase and a corresponding decrease in G1 phase. However, apigenin intervention before exposure to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone caused decreased expression of mutp53, cyclin D1, CDK4, and p-Rb and arrested the G1 phase (Yin et al., 2020). In all articles we encountered, the results showed that flavonoids have a favorable anti-HPV effect for certain chemical classes. Therefore, flavonoids have good potential in HPV treatment.

CONCLUSION

HPV infection and cervical cancer continue to be global and national health problems in Indonesia. This is reflected in the fact that cervical cancer lacks a definitive solution, with incidence rates continuing to increase annually. The prevention of cervical cancer using flavonoids shows promising potential as anti-HPV agents, with mechanisms varying according to the type of compound. Based on this literature review, the natural materials with the highest flavonoid content are raja banana peel, bisul fruit seeds, and matoa. Nevertheless, since these materials are difficult to obtain, shallot waste can serve as an alternative option.

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