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# Properties of vitexin, tiliroside and 5,7-dihydroxy-3,8,4'-trimethoxy flavonoids in the treatment of diseases of the female reproductive system: an *in silico* approach

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**Abstract.** Problems in the female reproductive system appear at the beginning of the reproductive cycle and persist until hormonal regulation in menopause. The drug therapies currently used can cause intolerance and susceptibility to other diseases, making it important to search for alternative therapeutic approaches that can be adopted during your reproductive life. Women use several species of plants to treat reproductive disorders. Some species are rich in bioactive molecules, such as flavonoids. The present study evaluated the bioactive in silico profile of three flavonoids: tyroside (Kaempferol-3-O-β-D- (6 ”-Ep-coumaryl) glucopyranoside), Pg-1 (5,7-dihydroxy-3,8, 4'-trimethoxy) and vitexin (5,7,4'-trihydroxyflavone-8-

C-β-glucopyranoside). The predictive antineoplastic action on organs of the female reproductive system and the protective activity against pathogens (protozoa, bacteria, fungi and viruses) were addressed. The study showed that vitexin had antiviral activity, tiliroside had a better index of antibacterial and antifungal activity and Pg-1 had antiprotozoal activity. The incidence of neoplasms in the female reproductive system is due to hormonal variation and injuries possibly caused by recurrent infections. Therefore, flavonoids, which are considered phytoestrogens, can regulate hormonal disorder and help fight pathogens. Pg-1 had a better breast, uterine and ovarian anticancer potential, while tiliroside had a greater anticancer effect on the cervical region. Vitexin, despite its lower potential compared to other substances, also demonstrated satisfactory breast and cervical cancer activity. Therefore, these molecules can become important candidates for natural drugs with antimicrobial and anticancer activity, contributing to the use of non-synthetic products.
**Keywords:** phytodrugs, *in silico*, flavonoids

## Introduction

Drug therapies used by women to treat gynecological infections can cause intolerance and increase susceptibility to other infectious diseases, making it important to search for alternative treatments for the conditions that affect these women during their reproductive life (Muresan et al., 2017). Popular medicine has a vast collection of information on the use of plants, teas, infusions, ointments and various forms of use and application of these natural products in the treatment of diseases of the female reproductive system (Pinto et al., 2009).

In the world scenario, about 130 to 175 million cases of urinary infections occur annually, with Brazil presenting 50% of the average of urinary infections registered among the cases evaluated in the gynecological area. *Escherichia coli* is the agent most commonly responsible for infections, being present in 80% of cases (Sousa et al., 2019). However, other microorganisms, such as bacteria, fungi, protozoa and viruses, are also considered agents responsible for causing various diseases in women.

The cervical-vaginal microbiota has lactobacilli that maintain the balance of the vaginal environment (ideal low pH), inhibiting the growth of other microorganisms. The interaction of the organism with substances present in food, hygiene products and drugs alters the pathophysiological process that the microbiota exerts on the female reproductive system. Therefore, the search for natural products that have pharmacological potential with less side effects and low cost is an alternative that is widely sought by women (Santos et al., 2018).

Helicteres velutina, Pavonia glazioviana and Waltheria viscosissima (Malvaceae), species native to the northeastern region of Brazil, are plants known for their antiprotozoal, antibacterial, antifungal activities, in addition to their anti-inflammatory action. Recent phytochemical studies have revealed the predominance of flavonoids among phytocomposites isolated in these species (Fernandes et al., 2020; Andrade et al., 2012; Cretton et al., 2016). These compounds provide protection against pathogens and are able to repair cell damage, improve the activity of enzymes in endothelial cells and reduce the generation of free radicals (Zhang et al., 2009).

The discovery of Brazilian natural species, applied in the treatment of diseases, contributes to the enrichment of the genetic and scientific heritage of the country. Therefore, the present study aimed to evaluate the bioactive potential in silico of flavonoids: tiliroside (Kaempferol-3-O-β-D- (6 ”-Ep-coumaryl) glucopyranoside), Pg-1 (5,7-dihydroxy -3,8,4'-trimethoxy), and vitexin (5,7,4'-trihydroxyflavone-8-C-β-glucopyranoside), addressing the antineoplastic predictive action on organs of the female reproductive system and protective activity against pathogens (protozoa, bacteria, fungi and viruses).

## Methods

*Chemical Structure*

Three flavonoids were selected for study: Tiliroside - Kaempferol-3-O-β-D- (6”-Ep-coumaril) glucopyranoside (Helicteres velutina), Pg-1 - 5,7-dihydroxy-3,8,4'- trimethoxy (Pavonia glazioviana) and Vitexin - 5,7,4'-trihydroxyflavone-8-C-β-glucopyranoside (Waltheria viscosissima), molecules present in species of the Malvaceae family that are native to Brazil. The chemical structures of the molecules were obtained through Pubchem® (https://pubchem.ncbi.nlm.nih.gov) (Figure 1).

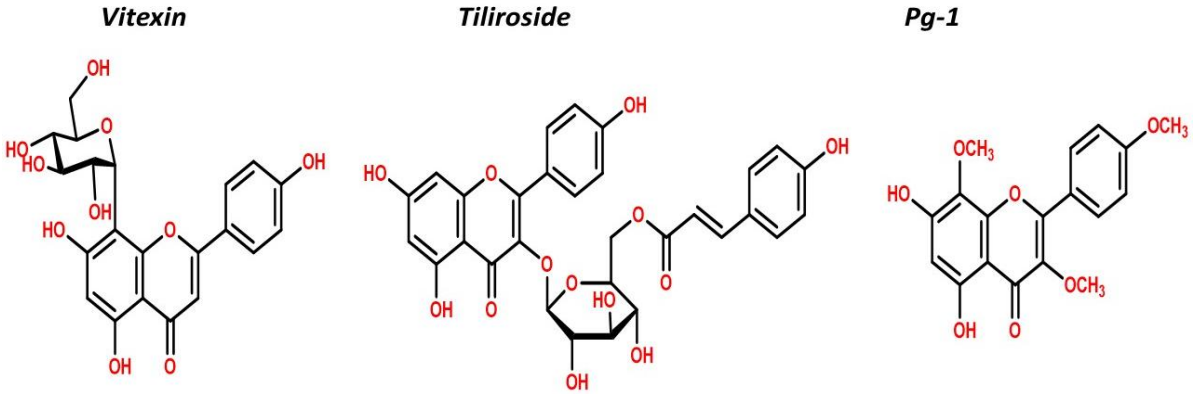


**Figure 1.** Flavonoids vitexin, tiliroside e *Pg*-1

*In silico analysis to predict the activity spectra of substances (PASS)*

The predictive bioactive properties were determined by the software PASS online® (http://www.pharmaexpert.ru/passonline/) a program designed to evaluate the biological potential of organic molecules in silico on the human organism, provides simultaneous predictions of several types of biological activities based on the structure of the compounds. Thus, it predicts the spectrum of activity of a compound as activation probability (Pa) or inactivation probability (Pi). The values of Pa and Pi are in the range of 0.00 to 1.00. When Pa is greater than Pi, the compound is considered to be experimentally active (Rakib et al., 2020).

## Results and discussion

Predictive analysis of the bioactive properties of vitexin, tiliroside and Pg-1 demonstrates that the molecules have a good probability of activation (Pa) in relation to the values of probability of inactivation (Pi) for different microorganisms (protozoa, bacteria, fungi and viruses) that cause infections in the female genital tract.

The present study revealed that Pg-1 had a better probability of activation in antiprotozoal activity, especially for *Trichomonas* (Pa: 0.241; Pi: 0.074), responsible for vaginal trichomoniasis contracted through sexual contact. Tiliroside had a better index of antibacterial activity (Pa: 0.616; Pi: 0.008) and a better antibiotic effect (Pa: 0.301; Pi: 0.014). Its action against mycoplasma hominis (Pa: 0.322; Pi: 0.014) and treponema pallidum (Pa: 0.145; Pi: 0.029), which are both bacteria related to urethritis and sexually transmitted diseases, such as syphilis, stands out. In addition, it has antifungal action (Pa: 0.765; Pi: 0.007) proving to be a potent substance against these microorganisms. Vitexin showed antiviral activity for Hepatitis B (Pa: 0.142; Pi: 0.041) and Herpes simplex (Pa: 0.479; Pi: 0.005), which can cause lesions in the genitourinary tract (Table 1).

**Table 1.** Probability of antimicrobial substances to act on the female reproductive system

| Antimicrobial Potential | Vitexin | | Tiliroside | | Pg-1 | |
|---|---|---|---|---|---|---|
| | Pa | Pi | Pa | Pi | Pa | Pi |
| Antiprotozoan | 0,321 | 0,039 | 0,426 | 0,016 | 0,433 | 0,015 |
| Antiprotozoan (*Trichomonas*) | --- | --- | --- | --- | 0,241 | 0,074 |
| Anti-bacterial | 0,541 | 0,013 | 0,616 | 0,008 | 0,391 | 0,032 |
| Broad spectrum antibiotic | 0,298 | 0,014 | 0,301 | 0,014 | 0,162 | 0,042 |
| Antimycoplasmal | 0,226 | 0,041 | 0,322 | 0,014 | 0,162 | 0,083 |
| Antispirochetal | 0,091 | 0,076 | 0,182 | 0,030 | 0,087 | 0,081 |
| Antitreponemal | 0,150 | 0,026 | 0,145 | 0,029 | 0,119 | 0,060 |
| Antifungal | 0,722 | 0,009 | 0,765 | 0,007 | 0,534 | 0,025 |
| Antifungal Enhancer | 0,113 | 0,034 | 0,100 | 0,067 | 0,121 | 0,022 |
| Antiviral | 0,360 | 0,021 | 0,319 | 0,030 | 0,217 | 0,082 |
| Antiviral (CMV) | --- | --- | --- | --- | 0,211 | 0,152 |
| Antiviral (Hepatitis) | 0,142 | 0,041 | --- | --- | 0,104 | 0,098 |
| Antiviral (Hepatitis B) | 0,479 | 0,005 | 0,422 | 0,012 | 0,463 | 0,007 |
| Antiviral (Herpes) | 0,753 | 0,002 | 0,539 | 0,006 | 0,436 | 0,022 |
| Antiviral (HIV) | 0,133 | 0,089 | 0,176 | 0,043 | --- | --- |

**Pa:** Probability of activation, **Pi:** Probability of inactivation.

The predictive analysis of Pg-1's antiprotozoal activity corroborates the findings in previous studies that analyzed chemical structures of several flavonoids and those with a greater number of hydroxyls in ring A, such as tiliroside and Pg-1, showed greater antiparasitic activity (Ramirez et al., 2010). However, specific *in vitro* and *in vivo* tests have not yet been performed to determine the mechanism of action of these molecules. In relation to tiliroside, extracts from African plants containing this flavonoid have been shown to have antibacterial and antifungal activity against sexually transmitted microorganisms, such as *Mycoplasma hominis*, *Staphylococcus aureus* and *Candida* sp. (Luhata & Luhata, 2017; Mongalo et al., 2017). These data corroborate the results described in the in silico analysis for tiliroside, indicating that this molecule, due to its natural properties, is a potential candidate in helping women's health.

The information obtained in the in silico test for vitexin supports the study carried out by Fahmy et al. (2020), which showed the good in vitro activity of the extract and fraction of *Erythrina speciosa* (containing vitexin) against viral species, such as Herpes simplex and Hepatitis A. These viruses can be transmitted by sexual contact and generate lesions in the genitourinary tract that promote the development of multiple infections or cancer. In addition, internal and external factors also contribute to the pathophysiological relationship of microorganisms from the resident vaginal microbiota, whose balance of this relationship can prevent or

favor recurrent infections and possible gynecological lesions (Veeh et al., 2003).

In women, the reproductive system undergoes several changes that are controlled by hormones produced in the hypothalamus, adenohypophysis and ovaries. Natural therapy with the use of phytoestrogens has been an important target for the treatment of female hormonal disorders, using flavonoids from plants as a tool to regulate, control and combat pathogens that affect women's health (Cheng et al., 2007; Cornwell et al., 2004). The chemical structures of flavonoids are composed of heterocyclic phenolic rings, similar to those of natural and synthetic estrogens. Due to the structural similarity of phytoestrogens with endogenous estrogens, they competitively bind to the cell receptor and naturally inhibit enzymes involved in hormonal metabolism (Figueiredo et al., 2011). Therefore, analyzes with predictive in silico study on the bioactivity of molecules found in natural sources are of great relevance to the collection of substances to be tested and which are biotechnologically relevant to the health of the population. The incidence of lesions in the female genitourinary system occurs mostly in early adulthood until the establishment of post-menopause. During this period, there is a greater fluctuation in the hormonal pattern and an increase in predisposing factors to trigger sudden changes in the microbiota and, consequently, in epithelial development during reproductive cycles (Sousa et al., 2019). Therefore, phytoestrogens, such as flavonoids, appear as a possible alternative for the regularization of hormonal disorders and to help fight pathogens.

Predictive analysis of the antineoplastic potential revealed that Pg-1 obtained better anti-cancer potential for uterine and ovarian cells. Tiliroside showed greater anticancer potential for the cervical region. Vitexin exhibited lower potentials than the substances previously tested, although satisfactory results have been described for the potential cervical anticancer (Table 2).

**Table 2.** Probability of antineoplastic substances to act on the female reproductive system

| Antineoplastic Potential | Vitexin | | Tiliroside | | Pg-1 | |
|---|---|---|---|---|---|---|
| | Pa | Pi | Pa | Pi | Pa | Pi |
| Cervical cancer | 0,325 | 0,016 | 0,366 | 0,012 | 0,297 | 0,019 |
| Uterine cancer | 0,145 | 0,039 | 0,123 | 0,081 | 0,181 | 0,015 |
| Ovary cancer | 0,164 | 0,051 | 0,234 | 0,029 | 0,335 | 0,015 |

**Pa:** Probability of activation, **Pi:** Probability of inactivation.

Recent studies show that the development of natural anticancer drugs has gained space in the midst of synthetic chemotherapeutic drugs, which are known to have greater toxicity and cause serious damage to healthy cells in the body. Theoretically, products of natural origin, with antioxidant and anti-inflammatory properties, could be used to replace synthetic anticancer drugs (Grochowski et al., 2018), which would make vitexin, tiliroside and Pg-1 flavonoids viable options in the fight against cancer. Several *in vitro* tests corroborate the results described for the molecules analyzed in this study: Rao et al. (2007) used tiliroside against intestinal, liver and skin cancer cells, Jahaniani et al. (2005) tested a flavonoid analog to Pg-1 in assays with fibrosarcoma cells, and Zhou et al. (2009) tested vitexin in ovarian and prostate cancer cells. All trials revealed the potential for inhibition and proliferation of cancer cells *in vitro*. However, the mechanisms of inhibition of each molecule in relation to the different types of cancer mentioned need more evidence to consolidate their mechanism of pharmacological action.

## Conclusion

The in silico analysis carried out in this study identified the probability of activity of the flavonoids vitexin, tiliroside and Pg-1 against pathogens that act in the female genitourinary tract: vitexin had antiviral activity, tiliroside had a better index of antibacterial and antifungal activity, and Pg-1 showed antiprotozoal activity. The evaluation of the probability of the antineoplastic potential of the substances showed that Pg-1 has a better uterine and ovarian anticancer potential, while tiliroside had a greater anticancer potential for the cervical region. Vitexin, despite exhibiting less potential than the other substances described in this study, demonstrated satisfactory cervical cancer activity. Thus, the studied molecules are promising candidates for future targets in the development of drugs with antimicrobial and anticancer activity for the female reproductive system, the performance of more detailed experiments may reveal the mechanism of action through *in vitro* and *in vivo* tests.

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