

Supporting the protective functions of the cervicovaginal epithelium and vaginal microbiota by witch hazel bark and its main constituent – hamamelitannin

Vaginal microbiota (VM) is a community of microorganisms inhabiting the lower part of the female genital tract, unique to each woman. Unlike the gut microbiota, where greater diversity is more beneficial to the host, the optimal VM should be dominated by a single species of the genus *Lactobacillus*, e.g. *L. crispatus* or *L. gasseri*. These bacteria, living in close association with the epithelium of the vagina and cervix, constitute a protective barrier against pathogens and irritating substances. The beneficial substances they release, such as lactic acid and bacteriocins, ensure appropriate pH, stimulate mucus secretion, mobilize the immune system and support the barrier functions of the epithelium. However, the composition of the VM is very susceptible to change, and the key factors influencing it include hormone levels depending on age and menstrual cycle, ethnicity, the condition of the immune system, infections and antibacterial and antifungal therapies, as well as lifestyle including personal intimate hygiene, stress, diet, stimulants and sexual behavior.

The state of vaginal dysbiosis, in which *Lactobacillus* loses its dominance in favor of pathogenic bacteria, affects almost 30% of women, and another 34% of women have VM with a non-optimal composition. Such disorders in the VM cause increased inflammation and permeability of the epithelium to pathogens and irritating substances, which in turn leads to serious side effects, such as an increased risk of sexually transmitted diseases, pelvic inflammatory disease, and even premature birth. If clinical symptoms occur, such as burning, itching, redness, vaginal discharge and unpleasant odor, bacterial or fungal vaginitis is most often diagnosed. Treatment comes down to the administration of antibiotics or antifungal drugs, the use of which is inevitably associated with recurrence of the infection and increased resistance of microorganisms. The imperfections of existing therapeutic standards, the lack of alternative therapies and the growing demand make it necessary to search for effective and safe therapeutic and preventive substances.

Natural products have been a source of inspiration in the search for effective therapies for centuries. In traditional medicine, plant substances rich in tannins were used to treat gynecological diseases such as infections, inflammation and vaginal discharge. These are high-molecular polyphenolic compounds known for their antidiarrheal properties. Scientific research conducted so far has shown their beneficial effect on the intestinal epithelium and the mucous membrane of the mouth and nose. They demonstrated anti-inflammatory properties, sealing the epithelium and inhibiting the adhesion of pathogens.

Witch hazel bark (*Hamamelidis cortex*) obtained from witch hazel (*Hamamelis virginiana* L.) is a substance often used in traditional medicine to treat infections and irritations of intimate areas. The main and characteristic chemical compound found in witch hazel bark extracts is hamamelitannin (2',5-di-*O*-galloylhamamellose). Preliminary studies have shown that both the extract and isolated hamamelitannin inhibit inflammation of skin cells - keratinocytes and fibroblasts. Additionally, hamamelitannin strongly supports the regeneration of injuries in an *in vitro* wound healing test.

The *in vitro* studies planned in the project include the effect of the extract and hamamelitannin on regeneration, permeability, integrity and inflammation of the epithelium caused by infection. In addition, the impact on the growth, metabolic activity and adhesion to the epithelium of microorganisms that characterize optimal and non-optimal VM will be examined. Then, the analyzes will be extrapolated to a 3D tissue model of the vaginal epithelium to verify the safety and effectiveness of using the extract and hamamelitannin in the prevention and treatment of intimate infections and their clinical symptoms.

Although vaginal dysbiosis and its adverse effects affect millions of women around the world, effective strategies for their prevention and treatment have not yet been developed. This is due to an unjustified and insufficient amount of research on women's intimate health, which has been neglected in preclinical research for years. Successful implementation of the submitted project will shed new light on therapeutic strategies aimed at preventing and supporting vaginal health.