Current Perspectives on Medicinal & Aromatic Plants

NSCI

# Bioapigyn® Herbal Ointment and Pessaries Compared to Acidosalus® Pessaries and Vaginal Probiotic in the Treatment of Vulvo-Vaginal Disorders

Višnja Oreščanin[1]*, Štefica Findri Guštek[2], Rosa Ferinčević[3], Ivan Fistonić[4], Damir Hodžić[5], Josip Valetić[5], Josipa Rodić[6]

[1]ORESČANIN Ltd., Laboratory for herbal drugs development, A. Jakšića 30, Zagreb, Croatia,
[2]Findri-Guštek Centre for healtcare, Ninska 5, Sesvete, Croatia
[3]MarinaLab Opus Ltd., Kraljevečka cesta 7, Kupinečki Kraljevec, Croatia
[4]Obstetric/Gynecology & Menopause Clinic, Preradovićeva 10, 10000 Zagreb, Croatia
[5]Merkur University Hospital, Zajčeva 19, 10000 Zagreb, Croatia
[6]Family medicine clinic, Vilima Korajca 19, 10000 Zagreb, Croatia

*Correspondence: vorescan@gmail.com, (Višnja Oreščanin)

Published: 29 July 2019

## Abstract

**Objective / Purpose:** The objective of the study was assessment of the clinical efficacy and safety of Bioapigyn® vaginal ointment and pessaries compared to Acidosalus® pessaries and vaginal probiotic in alleviating vulvo-vaginal disorders.
**Materials and methods:** 124 females were randomly selected into four groups (each of 31 participants) and treated once a day for ten days with: A) Bioapigyn® vaginal pessaries (one pessary per day); B) 2.5 mL of Bioapigyn® vaginal ointment; C) Acidosalus® vaginal pessaries (one pessary per day); D) 2.5 mL of Acidosalus® vaginal probiotic. All the patients were subjected to gynecological examination, measurement of vaginal pH, Pap test, native and KOH test, self-assessment and clinical assessment of the symptoms at baseline and following the therapy.
**Results:** Following the treatment with Bioapigyn® vaginal ointment, Bioapigyn® pessaries, Acidosalus® pessaries, Acidosalus® vaginal probiotic the total score of vulvo-vaginal disorders decreased for 85.2%, 88.9%, 82.7% and 78%, respectively and vaginal pH decreased between 9.1% and 14.3%. Native test showed normalization of the vaginal flora following the treatment with all four products with no significant difference among the products.
**Conclusion/Discussion:** Both Bioapigyn® and Acidosalus® products were highly efficient in alleviation of vulvo-vaginal disorders. The products created unfavorable conditions for pathogenic growth through the lowering of vaginal pH value, promoting the growth of lactobacilli, creating the environment with low water activity and creating a protective layer on the damaged mucosa that creates a physical barrier to the entrance of the pathogens into the cells and enables the recovery of the vaginal mucosa.

**Key words:** Bioapigyn, Acidosalus, vulvo-vaginal disorders, vaginal pH, herbal macerates, honey

## 1. Introduction

Abnormal vaginal discharge accompanied by itching, burning and inflammation together with vaginal pH >4.5 are the most common symptoms of vaginal infection in the reproductive age women (Larsen et al., 1993). Those symptoms are commonly associated with bacterial vaginosis (BV), trichomoniasis and/or candidiasis. In reproductive age female's high estradiol level promotes proliferation of vaginal epithelial cells and increase in their glycogen content. Subsequently, anaerobic metabolism of vaginal glycogen to

acetic and lactic acids by both vaginal lactobacilli and epithelial cells result with an acidic pH (3.6-4.5), necessary for maintaining healthy balance of the vaginal flora (Boskey et al., 1999).

Increase in vaginal pH represents significant trigger for increased growth of anaerobic bacteria (Klebanoff et al., 1990). Moreover, the lack of vaginal lactobacilli resulting in the lower production of hydrogen peroxide, and consequently, the increase in the concentrations of anaerobic bacteria (Redondo-Lopez et al., 1991; Panda et al., 2014). Mania-Pramanik et al., (2008.) suggested that pH of vaginal secretion could be used as a simple indicator of bacterial vaginosis in reproductive age women based on high correlation between increased vaginal pH and BV.

Due to lack of estradiol in perimenopausal and menopausal/postmenopausal women vaginal pH could increase significantly up to 7.5 (Milsom et al., 1993; Pandit i Ouslander, 1997; Cailloutte et al. 1997; Roy et al., 2004; Vahidroodsari et al., 2010; Panda et al., 2014) causing disturbance of healthy balance of the vaginal flora and creating the environment that supporting pathogens growth. Moreover, Tuntiviriyapun et al., 2015 found significant correlation between vaginal pH and vaginal atrophy in postmenopausal women accompanied with vaginal dryness and dyspareunia.

The purpose of this work was testing both clinical efficiency and safety of four medical devices in alleviating the symptoms of vulvo-vaginal disorders (vaginal discharge, odor, itching, burning, edema, vaginal dryness, dyspareunia) caused by increased vaginal pH in reproductive age women as well as those of perimenopausal/menopausal/ postmenopausal age based on their physical mode of action.

For that purpose, the following medical devices were used: Bioapigin® vaginal ointment, Bioapigyn® pessaries, Acidosalus® pessaries, Acidosalus® vaginal probiotic and their efficiencies were compared.

## 2. Material and Methods

***Study design:*** The study was designed as the prospective, randomized, controlled, comparative clinical trial. The study protocol was approved by the Ethics Committee of Findri Gustek Health Center with EudraCT number: 2019-001054-26. All the participants signed informed consent and completed the questioners.

***Patients:*** The inclusion criteria for recruitment to the study are as follows: (1) Nonspecific and mixed vulvo-vaginal disorders characterized by vaginal discharge and at least one additional sign/symptom; (2) Women older than 18 years of age; (3) Degree of vaginal purity ≤II; (4) Negative KOH test; (5) In the investigator's judgment, the patients should receive local treatment only; (6) vaginal pH > 4.5; (7) Signed informed consent.

*The exclusion criteria for both reproductive and non-reproductive participants were:* Trichomonas vaginalis infection (clinically suspected), Neisseria gonorrhoea infection (clinically suspected), Chlamydia trachomatis infection (clinically suspected), Infection with Herpes simplex virus (clinically suspected), Patients with other vaginal or vulvar conditions which would confound the interpretation of clinical response, Women with cervical carcinoma, Women under treatment for cervical intraepithelial neoplasia (CIN),

Recurrent bacterial vaginosis (three or more episodes of BV in 12 months), Recurrent vulvovaginal candidiasis (four or more VVC episodes in 12 months), Use of topical or systemic antimicrobial treatment within 30 days before the beginning of the study, On-going immunosuppressant treatment, On-going systemic anti-microbiological therapy, On-going use of vaginal probiotics, Women currently menstruating or expecting menstruation within 1 week, Pregnancy (excluded by pregnancy test), Lactation, Patients who gave birth or underwent gynaecological surgery 2 months before the beginning of the study, History of allergic reactions to the plants or to the excipients of the medical device, Participation in another clinical study, Patient is either unwilling or unable to use the investigational medical device.

The patients who met all inclusion criteria (124 of them) were randomly selected by nurse (based on the randomization code) into one of four treatment group (31 patients per group).

***Treatment:*** First group was treated ten days with 2.5 mL of Bioapigyn® vaginal ointment per day inserted deep into vagina before bedtime using the applicator and externally in thin layer for ten consecutive days. Second and third groups were treated ten days with Bioapigyn® and Acidosalus® pessaries, respectively by inserting one pessary deep into vagina before bedtime. Fourth group was treated with Acidosalus® vaginal probiotic also for ten days by inserting 2.5 mL of probiotic deep into vagina with appropriate applicator before bedtime. Follow-up period for all groups was from Day 13 to Day 30.

***Investigational products composition:*** Bioapigyn vaginal ointment® is homogeneous, greasy, viscous mass of characteristic herbal odor and olive green color composed of 20% of honey, 10% of beeswax (Cera flava), 9% of glycerin, 12% of the macerate of the marigold flowers (*Calendula offici*nalis L.), 12% of the macerate of the plantain leaves (Plantago major L.), 12% of the macerate of the chamomile flowers (*Matricaria chamomilla* L.), 6% of the macerate of the lavender flowers (*Lavandula officinalis* L.), 6% of the macerate of the yarrow flowers (*Achillea millefolium* L.), 6% of the macerate of the aerial part of sage (*Salvia officinalis* L.), 6% of the macerate of the lady's mantle leaves (*Alchemilla vulgaris* L.); essential oils: 0.5% of tea tree (*Melaleuca alternifolia*), 0.3% of thyme (*Thymus vulgaris* ct. thymol), 0.2% of oregano (*Origanum vulgare*).

Bioapigyn pessaries® are homogenous solids of characteristic herbal odor and light yellow color packed in blisters (3 g each). One box contains 10 pessaries. Bioapigyn® pessaries are composed of 54.5% hydrogenated coco-glycerides (Witepsol E75), 4.5% of Cera alba, 8% of the oil macerate of *Calendula officinalis* L., 8% of the oil macerate of *Plantago major* L., 8% of the oil macerate of *Matricaria chamomilla* L., 4% of the oil macerate of *Lavandula officinalis* L., 4% of the oil macerate of *Achilea millefolium* L., 4% of the oil macerate of *Salvia officinalis* L., 4% of the oil macerate of *Alchemilla vulgaris* L.; essential oils: 0.5% of *Melaleuca alternifolia*, 0.3% of *Thymus vulgaris* ct. timol, 0.2% of *Origanum vulgare*.

Acidosalus® pessaries are class I medical device composed of *Hypericum perforatum* oil, Cymbopogon martinii oil, *Melaleuca alternifolia* oil, Propolis, *Calophyllum inophyllum* oil, *Allium sativum* oil, *Lactobacillus acidophillus*, *Lactobacillus rhamnosus*, Beta 1,3 glucan, *Malus aceticum*, *Theobroma cacao* butter; *Cera alba*.

Acidosalus® vaginal probiotics is class I medical device composed of Cow's milk, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, beta-glucan.

***Statistical Analysis:*** For statistical evaluation Statistica 11.0 software package was employed. The number of the participant was calculated by Power analysis. With a moderate size effect (0.35), the power strength of 80% and the Type I error rate of 0.05 and four groups required sample size was 31 participants per group. The description of the treated population was done by basic statistics and frequency tables. Statistical significance was set to $p<0.05$ in all the tests performed. The differences in the mean values of pH prior and after the treatment as well as between control and experimental group were assessed by t-test.

## 3. Results and Discussion

***Description of the treated population***

***Bioapigyn® vaginal ointment group:*** The participants ranged from 19 to 79 years (48.7±17.0). Among them 26% are menopausal/postmenopausal women. Number of childbirth ranged from 0 to 4 (1.6±1.0) and number of miscarriages from 0 to 2 (0.1±0.4). Among 31 participants there were no alcohol or narcotics consumers while 32.3% of them identified themselves as smokers. According to professional qualifications majority of them (64.5%) finished secondary education. Among 31 participants 51.6% of them are married, 74.2% of them had one partner in the last 12 months, 90.3% of them use no contraception. Boiled food was predominated type of diet (87.1%). 35.5% of the patients take dietary supplements (mostly vitamins), 35.5% of them suffer from various types of allergies while the same percentage of the patients reported chronic illnesses (hypertension, diabetes, heart conditions). 16.1% of the patients were exposed to medical radiation (mostly X-rays), 9.7% of them take hormonal replacement therapy and 41.9% of them take the medications for their chronic conditions. Among the symptoms of vulvo-vaginal disorders stated by the patients, vaginal discharge and vaginal discharge combined with either vaginal odor or vaginal dryness prevailed.

***Bioapigyn® pessaries group:*** Age range of the patients treated with Bioapigyn® pessaries was between 21 and 74 years (42.8±13.8). Number of childbirth ranged from 0 to 4 (1.5±1.2) and number of miscarriages from 0 to 2 (0.2±0.5). Majority of the participants finished secondary education (77.4%). 67.7% of them was married and 93.5% of them had one partner in the last 12 months. 96.8% of the participants use no contraceptives. Similar to the previous group the prevailing diet type was boiled food. 26 of 31 participants are non-smokers. Three of them consume alcohol and two of them narcotics occasionally. 25.8% of the participants take some of the dietary supplements. Among them vitamins are used most commonly. 35.5% of the patients suffer from some type of allergy and 16.1% suffer from chronic diseases (hypertension, diabetes) and those five patients take their medication regularly. Only one patient takes hormonal replacement therapy and three patients were exposed to medical irradiation 30 days before the study. Vaginal discharge accompanied with vaginal odor (22.6%), followed by vaginal discharge (19.4%) were the most common symptoms of vulvo-vaginal disorders.

***Acidosalus® pessaries group:*** Age range of the patients treated with Acidosalus® pessaries was between 21 and 81 years (43.2±13.7). Number of childbirth ranged from 0 to 6 (1.5±1.5) and number of miscarriages from 0 to 1 (0.2±0.4). Majority of the

participants finished secondary education (74.2%). 67.7% of them was married and 87.1% of them had one partner in the last 12 months. 87.1% of the participants use no contraceptives. Similar to the previous two groups the prevailing diet type was boiled food (87.1%). 27 of 31 participants are non-smokers. Three of them consume alcohol occasionally and one participant regularly while two of them take narcotics occasionally. 25.8% of the participants take some of the dietary supplements. Among them vitamins and minerals are used most commonly. 19.4% of the patients suffer from some type of allergy and 12.9% suffer from chronic diseases. None of the patients take hormonal replacement therapy and 25.8% of the patients were exposed to medical irradiation 30 days before the study. 19.4% were on medication during the course of the study. Vaginal discharge (25.8%) and vaginal discharge accompanied with vaginal odor (19.4%) were the most common symptoms of vulvo-vaginal disorders.

***Acidosalus® vaginal probiotic group:*** Age range of the patients treated with Acidosalus® vaginal probiotic was between 23 and 56 years (43.5±8.9). Number of childbirth ranged from 0 to 5 (1.7±1.8) and number of miscarriages from 0 to 2 (0.2±0.5). Majority of the participants finished secondary education (80.6). 61.3% of them were married and 71.0% of them had one partner in the last 12 months. None of the participants use contraceptives. Similar to the previous three groups the prevailing diet type was boiled food (87.1%). 27 of 31 participants are non-smokers. One participant consumes alcohol regularly while none of them take narcotics. 9.7 % of the participants take some of the dietary supplements. 29.0% of the patients suffer from some type of allergy and 6.5% suffer from chronic diseases. None of the patients take hormonal replacement therapy and 19.4% of the patients were exposed to medical irradiation 30 days before the study. 6.5% were on medication during the course of the study. Vaginal discharge (29.0%) was the most common symptom of vulvo-vaginal disorders.

***Clinical efficiency and safety***

***Bioapigyn® vaginal ointment group:*** Mean values and standard deviations for the signs and symptoms of vulvo-vaginal disorders assessed by the patients at baseline and following ten days of the treatment with Bioapigyn® vaginal ointment, Bioapigyn® pessaries, Acidosalus® pessaries and Acidosalus® vaginal probiotic were presented in Table 1. Significant decrease of the score for all the symptoms as well as total score was observed following the treatment. Decrease of the initial score ranged from 78.9% (for burning) to 96.8% (for odor) while the total score decreased for 85.2% (from 5.9±2.5 to 0.9±1.1). Significant decrease of all signs/symptoms determined by the investigator (Table 2) was also found following the treatment. Complete disappearance of the symptoms like edema, erythema, excoriation and erosion was obtained while the mean value of the score for vaginal discharge and total score decreased for 87.5% and 94.1%, respectively. Significant decrease was also obtained for vaginal pH (Table 2) which dropped from 5.6±0.9 to 4.8±0.6 (14.3%). Moreover, among 20 patients with the results of native wet mount test (Table 3) rated as 2$^{nd}$ degree of vaginal purity at baseline, in 18 of them normal microbial flora (degree of vaginal purity = I) was found in native wet mouth preparation following the treatment. In only two patients' degree of purity remained unchanged. Following the treatment, clinical cure was obtained in 27 of 31 patients (87.1%) while in other four participants (12.9%) clinical improvement was observed (Table 4). None of the patients reported new symptoms or worsening of the existing symptoms. On the contrary, all 31 participants reported decrease compared to baseline of all the signs/symptoms of vulvo-vaginal disorders. The investigator also

obtained decrease of all the symptoms compared to baseline. In 26 patients with abnormal vaginal pH (>4.5) at baseline significant decrease was obtained following the treatment. The rest of the patients with normal vaginal pH at baseline experienced no changes after the treatment.

**Table 1.** Mean values ( $\bar{X}$) and standard deviations (SD) for the signs and symptoms of vulvo-vaginal disorders assessed by the patients at baseline and following ten days of the treatment with Bioapigyn® ointment, Bioapigyn® pessaries, Acodosalus® pessaries and Acidosalus® vaginal probiotic

| Signs/ symptoms | Bioapigyn® ointment | | Bioapigyn® pessarie | | Acodosalus® pessaries | | Acidosalus® probiotic | |
|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD |
| Vaginal discharge | 1.6±1.0 | 0.3±0.5* | 1.8±1.1 | 0.2±0.4* | 2.1±1.0 | 0.4±0.6* | 1.9±1.1 | 0.5±0.9* |
| Odor | 0.9±1.2 | 0.0±0.2* | 0.5±0.9 | 0.0±0.0* | 1.0±1.2 | 0.1±0.3* | 1.3±1.2 | 0.2±0.4* |
| Itching | 1.2±1.3 | 0.1±0.3* | 0.9±1.1 | 0.1±0.3* | 0.6±1.1 | 0.2±0.6* | 1.1±1.3 | 0.4±0.8* |
| Burning | 0.9±1.2 | 0.2±0.5* | 1.1±1.2 | 0.1±0.3* | 0.5±1.0 | 0.2±0.7* | 0.6±1.0 | 0.1±0.2* |
| Vaginal dryness | 1.3±1.3 | 0.2±0.6* | 0.7±1.2 | 0.1±0.4* | 0.5±0.9 | 0.1±0.6* | 0.7±1.2 | 0.2±0.5* |
| Total score | 5.9±2.5 | 0.9±1.1* | 4.9±2.3 | 0.5±1.0* | 4.7±2.3 | 0.8±1.3* | 5.4±2.8 | 1.2±1.2* |

***Bioapigyn® pessaries group:*** According to patients' self assessment (Table 1) after 10 days of the treatment with Bioapigyn® pessaries all the symptoms of vulvo-vaginal disorders decreased significantly including the total score which dropped from 4.9±2.3 to 0.5±1.0. The symptoms decrease ranged from 80.9% (vaginal dryness) to 100% (odor) while total score decreased 88.9%. Significant decrease of the signs/symptoms was also confirmed by principal investigator (Table 2).

**Table 2.** Mean values ( $\bar{X}$) and standard deviations (SD) for the signs and symptoms of vulvo-vaginal disorders determined by the investigator at baseline and following ten days of the treatment with Bioapigyn® ointment, Bioapigyn® pessaries, Acodosalus® pessaries and Acidosalus® vaginal probiotic

| Signs/ symptoms | Bioapigyn® ointment | | Bioapigyn® pessarie | | Acodosalus® pessaries | | Acidosalus® probiotic | |
|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD | $\bar{X}$±SD |
| Edema | 0.4±0.5 | 0.0±0.0* | 0.2±0.4 | 0.0±0.0* | 0.2±0.4 | 0.0±0.0* | 0.4±0.5 | 0.1±0.4* |
| Erythema | 0.4±0.5 | 0.0±0.0* | 0.2±0.4 | 0.0±0.0* | 0.1±0.3 | 0.0±0.0 | 0.3±0.4 | 0.0±0.0* |
| Excoriation | 0.0±0.2 | 0.0±0.0* | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.2 | 0.0±0.0 |
| Erosion | 0.1±0.3 | 0.0±0.0* | 0.0±0.2 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| Vaginal discharge | 0.8±0.4 | 0.1±0.3* | 0.9±0.6 | 0.0±0.0* | 0.9±0.3 | 0.1±0.2* | 0.8±0.4 | 0.3±0.5* |
| Total score | 1.7±1.0 | 0.1±0.3* | 1.1±0.8 | 0.03±0.2* | 1.2±0.5 | 0.1±0.3* | 1.5±1.0 | 0.3±0.5* |
| Vaginal pH | 5.6±0.9 | 4.8±0.6* | 5.4±0.8 | 4.8±0.6* | 5.5±0.9 | 5.0±0.6* | 5.4±0.8 | 4.9±0.7* |

Edema, erythema, erosion and vaginal discharge disappeared completely while total score dropped from 1.1±0.8 to 0.03±0.2 (97.2%). Vaginal pH dropped significantly following the treatment from 5.4±0.8 to 4.8±0.6 (11.1%). Among 24 patients with the results native wet mount test (Table 3) rated as 2nd degree of vaginal purity at baseline, in 20 of them normal microbial flora (degree of purity I) was found in native wet mouth preparation following the treatment. In only two patients' degree of purity remained unchanged. Following the treatment, clinical cure was obtained in 27 of 31 patients (87.1%) while in other four participants (12.9%) clinical improvement was observed (Table 4). None of the patients reported new symptoms or worsening of the existing symptoms. On the contrary, all 31 participants reported decrease compared to baseline of all the signs/symptoms of vulvo-vaginal disorders.

***Acidosalus® pessaries group:*** According to patients' self assessment (Table 1) after 10 days of the treatment with Acidosalus® pessaries all the symptoms of vulvo-vaginal disorders decreased significantly including the total score which dropped from 4.7±2.3 to 0.8±1.3. The decrease of the symptoms ranged from 55.8 % (burning) to 90% (odor) while total score decreased for 82.7%. Significant decrease of the signs/symptoms was also confirmed by principal investigator (Table 2). Edema and erythema disappeared completely while vaginal discharge and the total score dropped from 0.9±0.3 to 0.1±0.2 and from 1.2±0.5 to 0.1±0.3, respectively. Vaginal pH dropped significantly following the treatment from 5.5±0.9 to 5.0±0.6 (9.1%). Among 24 patients with the results native wet mount test (Table 3) rated as 2nd degree of vaginal purity at baseline, in 21 of them normal microbial flora (degree of purity I) was found in native wet mouth preparation following the treatment. In only three patients' degree of purity remained unchanged.

**Table 3.** Degree of vaginal purity determined by native wet mouth test at baseline and following ten days of the treatment with Bioapigyn® ointment, Bioapigyn® pessaries, Acodosalus® pessaries and Acidosalus® vaginal probiotic

| Signs/symptoms | Bioapigyn® ointment | | Bioapigyn® pessarie | | Acodosalus® pessaries | | Acidosalus® probiotic | |
|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| I | 35.5 | 93.5 | 22.6 | 93.5 | 22.6 | 90.3 | 19.4 | 93.5 |
| II | 64.5 | 6.5 | 77.4 | 6.5 | 77.4 | 9.7 | 80.6 | 6.5 |

Following the treatment, clinical cure was obtained in 27 of 31 patients (83.9%) while in other five participants (16.1%) clinical improvement was observed (Table 4).
None of the patients reported new symptoms or worsening of the existing symptoms. On the contrary, all 31 participants reported decrease compared to baseline of all the signs/symptoms of vulvo-vaginal disorders.

**Table 4.** Clinical efficiency of Bioapigyn® ointment, Bioapigyn® pessaries, Acodosalus® pessaries and Acidosalus® vaginal probiotic

| Clinical efficiency | Bioapigyn® ointment | Bioapigyn® pessarie | Acodosalus® pessaries | Acidosalus® probiotic |
|---|---|---|---|---|
| Cure | 87.1 | 87.1 | 83.9 | 51.6 |
| Improvement | 12.9 | 12.9 | 16.1 | 48.4 |

***Acidosalus® vaginal probiotic group:*** According to the patients' self assessment (Table 1) after 10 days of the treatment with Acidosalus® vaginal probiotic all the symptoms of vulvo-vaginal disorders decreased significantly including the total score which dropped from 5.4±2.8 to 1.2±1.2. The decrease of the symptoms ranged from 69 % (itching) to 90.2% (burning) while total score decreased 78%. Significant decrease of the signs/symptoms was also confirmed by principal investigator (Table 2). Edema and erythema disappeared completely while vaginal discharge and the total score dropped from 0.8±0.4 to 0.3±0.5 and from 1.5±1.0 to 0.3±0.5, respectively. Vaginal pH dropped significantly following the treatment from 5.4±0.8 to 4.9±0.7 (9.3%). Among 25 participants with the results of native wet mount test (Table 3) rated as 2$^{nd}$ degree of vaginal purity at baseline, in 23 of them normal microbial flora (degree of purity I) was found in native wet mouth preparation following the treatment. In only two patients degree of purity remained unchanged. Following the treatment, clinical cure was obtained in 16 of 31 patients (51.6%) while in other 15 participants (48.4 %) clinical improvement was observed (Table 4). None of the patients reported new symptoms or worsening of the existing symptoms. On the contrary, all 31 participants reported decrease compared to baseline of all the signs/symptoms of vulvo-vaginal disorders.

***Comparison of the treatment efficiency:*** Following the treatment with Bioapigin® vaginal ointment, Bioapigyn® pessaries, Acidosalus® pessaries, Acidosalus® vaginal probiotic the mean value of the percentage of the total score of the patient's rate of vulvo-vaginal disorders decreased for 85.2%, 88.9%, 82.7% and 78%, respectively. There was no significant difference among four tested groups in the decrease of the total score of self-assessed vulvo-vaginal symptoms. However, significant difference was observed for burning between Acidosalus® pessaries and Bioapigyn® pessaries (p=0.0114) as well as between Acidosalus® pessaries and Acidosalus® vaginal probiotic (p=0.0064). The percentage of the rate of vulvo-vaginal disorders assessed by the investigator decreased for 94.1%. 97.2%, 91.7% and 80% following the treatment with Bioapigyn® vaginal ointment, Bioapigyn® pessaries, Acidosalus® pessaries, Acidosalus® vaginal probiotic, respectively. Significant difference was observed for edema decrease between Acidosalus® vaginal probiotic and other three products (p=0.0095), vaginal discharge decrease between Acidosalus® vaginal probiotic and Acidosalus® pessaries (p=0.0358), Bioapigyn® vaginal ointment (p=0.0462) and Bioapigyn® pessaries (p=0.0006). In the case of the total score significant difference was observed between Acidosalus® vaginal probiotic and Bioapigyn® pessaries (p=0.0277). Vaginal pH dropped for 14.3%, 11.1%, 9.1% and 9.3% after the treatment with Bioapigyn® vaginal ointment, Bioapigyn® pessaries, Acidosalus® pessaries, Acidosalus® vaginal probiotic. Although, Bioapigyn® vaginal ointment showed the best performance there was no significant difference among four tested groups that was confirmed by Newman-Keuls test. All four products received high rating by the patients (Table 5). The highest grade was received for Bioapigyn® pessaries and the lowest for Acidosalus® vaginal probiotic. The results of Newman Keuls test confirmed statistically significant difference between Acidosalus® vaginal probiotic and other three products.

Clinical cure rate was 87.1% for Bioapigyn ointment and pessaries, 83.9% for Acidosalus® pessaries and 51.6% for Acidosalus® vaginal probiotic. Significant difference was observed between Acidosalus® vaginal probiotic and Bioapigyn® ointment and pessaries (p=0.0066) as well as between Acidosalus® vaginal probiotic and Acidosalus® pessaries (p=0.0152).

**Table 5.** Mean values and standard deviations for the patient's grade of the tested products following ten days of the treatment. BP- Bioapigyn® pessaries; BO - Bioapigyn® vaginal ointment; AP – Acidosalus® pessaries; AVP – Acidosalus® vaginal probiotic

| Investigational product | $\bar{X}$ | SD |
|---|---|---|
| BP | 4.8 | 0.4 |
| BO | 4.7 | 0.6 |
| AP | 4.7 | 0.4 |
| AVP | 4.4 | 0.8 |

## 4. Discussion and Conclusions

***Bioapigyn® vaginal ointment:*** Ten days of the application of Bioapigyn® vaginal ointment resulted in 85% reduction of self assessed symptoms, 94% reduction of the signs/symptoms determined by Principal investigator and 14.4% reduction of vaginal pH which was the most important parameter in the maintenance of healthy balance of the vaginal flora. Moreover, the patients with disturbed balance of vaginal flora (2$^{nd}$ degree of vaginal purity determined by native wet mouth test) showed complete normalization and establishment of a healthy balance of the vaginal flora following the treatment. It was clinically confirmed that Bioapigyn® vaginal ointment reduced vaginal pH significantly which resulted in normalization of the vaginal flora and disappearance of the symptoms of vulvo-vaginal disorders with clinical cure rate of 87%. Significant decrease of the symptoms of all vulvo-vaginal disorders could be explained by decreasing of vaginal pH value and the establishment of normal acidic vaginal milieu unfavorable for the growth of pathogenic microorganisms. The ingredients, honey (pH = 4.16) and herbal macerate (pH = 5.82) causing acidic reaction and those components were responsible for the restoration of acidic vaginal pH and normalization of the existing vaginal flora. Moreover, high osmotic effect of honey resulted with creation of the environment with low water activity unfavorable for growth and multiplication of the pathogens that causes unpleasant odor, burning, itching and vaginal discharge. In other words, honey expressed its antimicrobial activity through the physical suction of water from the bacterial/fungal cells. Consequently, the application of the Bioapigyn® vaginal ointment with acidic reaction (pH of the ointment = 4.9 due to the presence of honey and herbal macerates) and high osmolarity (due to the presence of honey) resulted in the disappearance of unpleasant vaginal odor and vaginal discharge.

Moreover, 26% of the tested populations were menopausal and postmenopausal women that due to lack of the circulating estrogen suffering from vaginal dryness, itching, burning, irritation, painful intercourse. The application of the Bioapigyn® vaginal ointment with emollient effect due to the presence of glycerine and honey as well as the herbal macerate resulted in alleviation of the symptoms of vaginal dryness and associated symptoms like itching and burning in the vulvo-vaginal area. High viscosity and greasiness of the product provided excellent coating of the mucosa and enabled its recovery while simultaneously prevented irritation and pain during sexual intercourse. The combination of glycerine, honey, beeswax and the herbal macerate in the product with excellent coating effect resulted in disappearance of the symptoms like vaginal dryness, itching, burning, and edema. Moreover, the product crated the protective layer on the damaged vaginal mucosa, and prevented the adhesion of pathogens. Low pH, high

osmolarity, high viscosity and greasiness, emollient, humectants as well as low water activity of Bioapigyn® vaginal ointment resulted in significant decrease of the symptoms of vulvo-vaginal disorders due to: the creation of unfavorable conditions for the growth, adhesion and multiplications of the pathogens, the creation of the protective coating on the vaginal mucosa enabling its recovery and preventing further irritation, alleviation of the vaginal dryness due to the presence of the humectants, preventing the pain during intercourse due to lubricating effect.

Previous studies also showed beneficial effect of honey alone or in combination with other ingredients in the treatment of vulvo-vaginal disorders. 70 non-pregnant women with Candidal vulvovaginitis were treated seven days with either vaginal cream consisted of honey and yogurt (N = 35) or with clotrimazole vaginal cream (N = 35). Following the treatment a significant decrease of all the symptoms was observed in both control (from 71% to 90%) and „yogurt and honey" (from 83% to 97%) group. When comparing two groups significantly better results in the case of itching, irritation and vaginal discharge were observed in „yogurt and honey" treatment group (Darvishi et al., 2015). 129 pregnant women with Vulvovaginitis candidiasis were treated with honey and yogurt mixture or with itraconazole resulted in significant improvement of the symptoms (87.8% and 72.3%, respectively). The symptoms like itching, secretions, and redness of the vulva and vagina, has significantly decreased in honey and yogurt based cream compared to itraconazole group (Abdelmonem et al., 2012). Eighty women diagnosed with vulvovaginal candidiasis were treated seven days with either honey ointment or clotrimazole ointment. The symptoms including inflammation, vaginal discharge, and irritation at baseline in the fourth and eighth days of the treatment were examined and compared between the two groups (Banaeian et al., 2017). In both groups, all the symptoms disappeared after the treatment. On the eighth day of the treatment, there was a significant difference in inflammation, irritation and vaginal discharge as well as treatment satisfaction between two groups. No side effects were reported in either group. In the clinical study conducted by Oreščanin and Findri Guštek (2017.) 80 female patients with positive swabs to at least one microorganism (U. urealyticum, M. hominis, *E. coli* and *Candida* sp.) as well as the symptoms like vaginal discharge, irritation, burning, unpleasant odor, vaginal dryness was randomly divided into three treatment groups. First group was treated 12 days (twice a day) with doxycycline antibiotic and 2 g of Bioapigyn® vaginal ointment (once a day), second group with 2 g of Bioapigyn® ointment only and third group with antibiotic only (12 days; twice a day). Following the treatment all the swabs were negative to M. hominis regardless of the treatment group. Eradication of *U. urealyticum* was 100%, 87% and 62% and E. coli 75%, 67% and 33% in antibiotic + herbal ointment, herbal ointment only and antibiotic only group, respectively. None of the patients in the first group developed antibiotic-associated candidiasis compared to 80% positive swabs in the antibiotic only group. The symptoms like vaginal discharge, irritation, and burning, unpleasant odor, vaginal dryness disappeared completely in both antibiotic+ ointment and ointment only treatment groups. The authors concluded that Bioapigyn® vaginal ointment has antimicrobial potential against common vaginal pathogens and is highly effective in the prevention of antibiotic-associated yeast infection which was linked with low pH of the ointment as well as osmotic effect of honey that created the environment unfavorable for most common vaginal pathogens. Based on the results of the current study and the previously published data it could be concluded that Bioapigyn® vaginal ointment is safe and clinically efficient in alleviating the symptoms of

vulvo-vaginal disorders by physical mode of action due to coating, pH adjusting, osmotic, moisturizing and lubricating effect.

***Bioapigyn® prssaries:*** Bioapigyn® pessaries exhibited even better results in the treatment of vulvo-vaginal disorders with 89% decrease of self assessed symptoms, 97.2% decrease in the signs/symptoms determined by the investigator, 11.1% of decrease in the vaginal pH. The patients with disturbed balance of vaginal flora (2nd degree of vaginal purity determined by native wet mouth test) showed complete normalization and the establishment of healthy balance of the vaginal flora following the treatment. It was confirmed clinically that Bioapigyn® pessaries reduced significantly vaginal pH and created the environment with no water activity which resulted in normalization of the vaginal flora and disappearance of the symptoms of vulvo-vaginal disorders with clinical cure rate of 87%.

Excellent coating, emollient and lubricating effect alleviated vaginal dryness and protected vaginal mucosa from irritation. Moreover, high viscosity and greasiness of the product enabled recovery of the vaginal mucosa while simultaneously prevented irritation and pain during sexual intercourse. This mode of action could be linked to the following ingredients: (a) hydrogenated coco-glycerides (excellent coating, emollient, high viscosity, greasiness and moisturizing effect); (b) beeswax (excellent coating effect; creating environment with no water activity); (3) sunflower oil macerates of the plants (*Calendula officinalis* L.), plantain leaves (*Plantago major* L.), chamomile flowers *(Matricaria chamomilla* L.), lavender flowers (*Lavandula officinalis* L.), the areal parts of yarrow flowers (*Achillea millefolium* L.), sage leaves (*Salvia officinalis* L.), lady's mantle leaves (*Alchemilla vulgaris* L.) (low pH, coating, emollient, high viscosity, greasiness, creating environment with no water activity); (4) essential oils: Melaleuca alternifolia, *Thymus vulgaris* ct. thymol and *Origanum vulgare* (deodorizing effect, natural preservation effect).

***Acidosalus® suppositories and vaginal probiotic:*** Ten days of the application of Acidosalus® pessaries and Acidosalus® vaginal probiotic resulted in 82.5% and 78% reduction of self assessed symptoms, 94.4% and 80% reduction of the signs/symptoms determined by Principal investigator and 9.1% and 9.3% reduction of vaginal pH. In addition to that, the patients with disturbed balance of vaginal flora (2$^{nd}$ degree of vaginal purity determined by native wet mouth test) showed complete normalization and establishment of a healthy balance of the vaginal flora after the products application. Low pH, probiotic and coating effect of Acidosalus® pessaries and Acidosalus® vaginal probiotic resulted in normalization of vaginal flora and disappearance of the symptoms of vulvo-vaginal disorders with clinical cure rate of 83.9% and 51.6%, respectively.

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