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# Application of new Herbal Pessaries for the Treatment of the Lower Genital Tract Infections

KEYWORDS: herbal pessaries, U. urealyticum, olive oil extracts, medicinal plants, essential oils


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**ABSTRACT** *The main purpose of this work was to test the efficiency of newly developed herbal pessaries to prevent antibiotic-associated yeast infection during the treatment of Ureaplasma urealytium with doxycycline. Besides, the influence of the pessaries on the efficacy of the treatment of other bacterial, yeast and viral infections as well as low grade cervical intraepithelial lesions was also assessed. 93 women in the age range from ranged 19 to 64 years (34.4±9.3) represented experimental group and 97 women ranging from 22 to 60 years (32.7±6.3) represented control group, both infected with Ureaplasma urealytium were included in the study. The patients from the experimental group were treated with doxycycline for 12 days and locally for 10 days with new herbal pessaries while the control group was treated with doxycycline and gyno-daktarin pessaries. Significantly higher efficiency was obtained for the experimental group ($x^2$=8.39; P=0.0038) in eradication of U. urealytium (91.5%) compared to the control group (69.1%). Besides, complete eradication of M. hominis and C. trachomatis was also observed in the experimental group. All the swabs were negative to Candida sp. following the treatment with herbal pessaries compared to 26.8% positive swabs in the case of gyno-daktarin treatment. A significant improvement was also observed in the results of Pap test following the treatment with new herbal pessaries ($x^2$ = 23.3; P < 0.0000). Among the predictor variables, the type of the therapy had the highest, statistically significant influence on the outcome of the therapy. The synergistic effect of the medicinal plants with well known wound healing, anti-inflammatory, antimicrobial and antiviral properties could be responsible for high treatment potential of the pessaries. This new product is superior to miconazole in the prevention of antibiotic-associated yeast infection.*

## INTRODUCTION

*Mycoplasmas* are obligate parasites of humans and animals that could be found in the oral mucosa, upper respiratory tract as well as genital tract adhered on the epithelial cells. Once adhered on the host cell they secrete metabolic products (most probably hydrogen peroxide) that destroy both host cells as well as bacteria itself (Taylor-Robinson and Bebear, 1997).

*Mycoplasmas* as ubiquitous microorganisms can be either pathogenic to humans or commensal, living innocuously with their host as part of the natural flora. The following *Mycoplasmas* are most commonly found in the urogenital tract: *M. hominis*, *M. fermentans*, *M. genitalium*, *M. ladlawi*, *Ureaplasma urealyticum* and *Ureaplasma parvum* (Klenic, 2001; Waites et al., 1999)

The incidence of *U. urealyticum* in the cervix or vagina of sexually active woman could be up to 80%. Higher prevalence was connected to younger age, freequent change of partners, lower socio-ecomomic status, use of oral contraceptives. In most cases the infection is asimptomatic (Cassell et al., 1993). Among the symptoms the most common are infertility, higher incidence of spontaneous abortion, pelvic pain, premenstrual symptoms like spotting, the symptoms of infection of the genital tract as well as itching, burning and vaginal discharge. Among the general symptoms chronic sore throat, kidney stones, red eyes, sensitivity to light, headaches, arthritis, asthma, abdominal pain, bloody stool, diarrhea are most common. *Ureaplasma* infection can also cause nonspecific urethritis. *Ureaplasma* infection is especially dangerous during the pregnancy because it can cause an infection of chorioamnionitis which could lead to premature spontaneous labor and delivery. The newborns could be infected by passing through the birth canal that could cause septicemia, meningitis, and pneumonia. The infection of the lower respiratory tract can lead to development of chronic lung disease in very low-birth-weight of infants (Cassell et al., 1993).

Since *Mycoplasmas* are lacking cell walls the best treatment solution is doxycycline antibiotic (Karabay et al., 2006). Although, highly efficient in eradication of genital patogens, the major drawback of the treatment is frequent development of severe candidiasis.

The purpose of this work was testing of new herbal based pessaries consisted of oil based extracts and essential oils of medicinal plants with well known wound healing, anti-inflammatory, antimicrobial and antiviral properties for the prevention of antibiotic-associated yeast infection as well as additional therapy for eradication of bacterial/yeast/viral infections of the female lower genital tract. The results were compared with those obtained by conventional treatment by miconazole based pessaries.

Oil based extracts of the plants *Calendula officinalis*, *Matricaria chamomilla*, *Lavandula officinalis*, *Hypericum perforatum* L., *Achilea millefolium*, *Thymus serpyllum*, *Salvia officinalis*, *Mentha piperita* L., *Symphytum officinale*, *Plantago major* L., *Alchemilla vulgaris* were used as an active components of the pessaries.

*Calendula officinalis* showed high potential in the induction of wound healing and re-epithelization (Chandran and Kuton, 2008; Preethi and Kutton, 2009). A significant anti-inflammatory effect (Preethi et al., 2009) was also noticed. Antibacterial activity of *C. officinalis* against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* was reported by Roopashree et al., 2008. *In vitro* antibacterial activity *of C.*

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*officinalis* aganist both gram positive (*S. aureus*, *B. subtilis*) and gram negative bacteria (*E. coli* and *K. pneumoniae*) was also reported (Chakraborthy, 2008). Gazim te al., 2008 confirmed excellent antifungal activity of *C. officinalis* against clinical isolates of *Candida* species with the inhibition zone diameter up to 30 mm. Similar results for *C. albicans* were also observed by Jansen et al, 1986. Antiviral properties of *C. officinalis* were reported by Muley et al., 2009.

*M. chamomilla* is well known medicinal plant with multitherapeutic properties. Among them anti-inflammatory, antiseptic, antiplogistic, and spasmolytic properties (Singh et al., 2011) are most common. Significantly higher wound closure percentage compared to the control group was reported following the treatment with *M. chamomilla* oil extract (Jarrahi, 2008; Jarrahi et al., 2010). The treatment of the tongue wound with *M. chamomilla* extract resulted in significantly higher, time dependent re-epithelization and percentage of collagen fibers (Duarte et al., 2011). The *M. chamomilla* based treatment of the ulcer resulted in significantly faster wound healing compared to the control (Martins et al., 2009). Significantly higher percentage of wound closure, faster re-epithelization, higher wound-breaking strength and higher hydroxyproline content compared to negative control (Nayak et al., 2007) was assessed following the treatment with *M. chamomilla* extract.

*Lavandula officinalis* was used in both folk and official medicine for wound healing as well as a disinfectant, antispasmodic, carminative, cholagogue, diuretic, stimulant and sudorific and flavouring agent (Bayoub et al, 2010). Sosa et al., 2005 reported a significant, dose dependent anti-inflammatory effect of lavender extract which was comparable to that of indomethacin. Both anti-inflammatory and analgesic effect of *L. angustifolia* was reported by Hajhashemi et al., 2003. Stanojević et al., 2011 reported good antimicrobial activity of *L. officinalis* against *S. enteritidis, K. pneumoniae, E. coli, S. aureus, E. faecalis, P. aeruginosa, C. albicans* and *A. niger* with growth inhibition zone ranging from 20 mm to 29 mm. Strong antimicrobial effect of the lavender against *S. aureus*, *P. aeruginosa*, *Candida* sp and *A. niger* was also reported **(**Kunicka-Styczyńska et al., 2009). Antibacterial activity of *L. officinalis* was also confirmed by Rostami et al., 2012. *L. officinalis* essential oil showed a significant antifungal activity against filamentous fungi (Larrondo et al., 1995).

Olive oil extract of *Hypericum perforatum* showed excellent wound healing and anti-inflammatory effect in dose-dependent manner (Süntar et al, 2010). A dose dependent anti-inflammatory activity of *H. perforatum* was also reported by other researchers (Sosa et al., 2007; Hammer et al., 2007; Abdel-Salam, 2005; Menegazzi et al., 2006; Zdunić et al., 2009; Tedeschi et al., 2003; Öztürk et al., 2007). Numerous authors reported antimicrobial activity of various extract of *H. perforatum* (Reichling et al., 2001). Excellent antibacterial activity of *H. perforatum* and its constituents was observed against gram-positive bacteria including penicillin-resistant (PRSA) and meticillin-resistant (MRSA) *S. aureus* (Schempp et al., 2003; Schempp et al., 1999). Antimicrobial activity against Gram-positive bacteria, *B. subtilis* and *B. cereus* was also reported by Avato et al., 2004. Meral and Karabay, 2002 reported antimicrobial activity of *H. perforatum* against Gram-positive (*S. aureus*, *S. epidermidis*, *E. faecalis*) and Gram-negative (*P. aeruginosae*, *E. cloacae* and *E. coli*) species which was comparable or better compared to standard antibiotics. Antiviral activity of *H. perforatum* was reported by Serkedjieva et al., 1990.

Sagdic, 2003 reported, both bacteriostatic and bactericidal activity of *Thymus serpyllum* against various strain of *E. coli*, *S. aureus* and *Y. enterocolitica*. The antimicrobial activity comparable or better than standard antibiotics (Ur Rahman and Gul, 2003) was observed against both, gram positive (*B. megaterium*, *B. subtilis*, *L. acidophilus*, *M. leuteus*, *S. albus*, *S. aureus*, *V. cholera*) and gram negative bacteria (*E. coli*, *S. typhimurium*, *S. ferrarie*).

A strong bacteriostatic and bactericidal activity of *Salvia officinalis* extract against *S. aureus* with MIC value of 60 µg/mL was reported by Snowden et al., 2014. *S. officinalis* showed strong antibacterial activity against *B. subtilis* and *S. aureus* (Balouiri et al., 2014) and strong synergistic effect with amoxicillin against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and clinical isolates *S. aureus*, *B. subtilis*, *E. cloacae*, *K. pneumoniae*, *E. coli* and *P. mirabilis*. Anti-inflammatory effect *S. officinalis* was also documented (Rodrigues et al., 2012; Oniga et al., 2007).

*Achillea millefolium* L. is one of the most widely used medicinal plants in the world, primarily for wound healing, digestive problems, respiratory infections, and skin conditions. Preclinical studies indicate that it may have anti-inflammatory, anti-ulcer, hepatoprotective, anxiolytic, and perhaps antipathogenic activities (Applequist and Moerman, 2011). Oil extract showed antimicrobial activity against *S. pneumoniae*, *C. albicans*, *M. smegmatis*, *A. lwoffii* and *C. krusei* (Candan et al., 2003). A significant antimicrobial activity was also reported against *S. aureus, E. coli, K. pneumoniae, P. aeruginosa, S. enteritidis, A. niger and C. albicans* (Stojanović et al., 2005).

*Menta piperita* essential oil showed strong antimicrobial activity against multiresistant strain of *Shigella sonei* and *Micrococcus flavus*. Besides, significant fungistatic and fungicidal activity against *Trichophyton tonsurans* and *Candida albicans* was also observed which was higher compared to bifonazole fungicide (Mimica-Dukić et al., 2003).

*Plantago major* have been used for centuries for wound healing, and also as analgetic, antioxidant, weak antibiotic, antiviral, antifungal, and anti-inflammatory agent, immuno modulator as well as antiulcerogenic (Samuelsen, 2000; Reina et al., 2013). Significantly better re-epithelization and significantly earlier wound closure was observed following the treatment with *P. major* based oinment compared to the control group (Thome et al., 2012). Wound healing potential of *Plantago major* was also demonstrated by Zubair et al., 2012 and Velasco-Lezama et al., 2006 with significant increase of epithelial cells proliferation compared to the negative control.

*Alchemilla vulgaris* is often used in folk medicine especially for the treatment inflammations, bleeding as well as various gynecological disorders. Wound-healing properties of *A. vulgaris in vitro* and *in vivo* was confirmed by Shrivastava et al., 2007 by increasing cell proliferation and decreasing the lesion diameter. The topical application *A. vulgaris* extract to the ulcers resulted in complete healing in 75% of the patients following the 3 day treatment (Shrivastava and John, 2006).

The benefitial effect of *Symphytum officinale* to wound healing could be directly linked with its phytochemical composition especially to its component allantoin (Staiger, 2012). Following the treatment of 161 patients with decubitus ulcers with *Symphytum* based cream during four

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weeks resulted in complete healing of the pressure sores in 85.9% of the patients and reduction of the total decubitus area for 89.2% (Stepán et al., 2014). *S. officinale* was found highly effective in wound healing in animle model with collagen deposition increase up to 240% and reduction of cellular inflammatory infiltrate up to 46% (Araújo et al., 2012). The wound healing mechanism induced by allantoin occurs via the regulation of inflammatory response and stimulus to fibroblastic proliferation and extracellular matrix synthesis (Araújo et al., 2010). A significant wound healing potential of the topically applied preparation Traumaplant® containing 10% active ingredient from medicinal comfrey was confirmed on the patients with fresh abrasions (Barna et al., 2007; Barna et al., 2012).

Among the essential oils those with well known antimicrobial properties were selected in our study.

*Melaleuca alternifolia* essential oil showed excellent antifungal activity against filamentous fungi associated with invasive fungal wound infections (Homeyer et al., 2015) and could be safely used for topical wound treatment. It was superior to fluconazole in growth inhibition of *C. albicans* (Dalwai et al., 2014) including fluconazole resistant strains (Vazquez et al., 2000; Ergin i Arikan, 2002) with MIC ranging from 0.25-4% and inhibition zone diameter ranging from 14 to 42 mm. Its antifungal activity was also confirmend against yeasts (*Candida* spp., *S. pombe*, *D. hansenii*) with the concentration ranged from 0.12% to 0.50% (v/v) (D'Auria et al., 2001). Tea tree oil at the concnetration of 0.25% and higher inhibited significantly germ tubes formation by *C. albicans* (Hammer et al., 2000). It was found effective against 57 *Candida* isolates with MIC90 of 0.5% (v/v) (Hammer et al., 1998). Comparable MIC concentrations were also obtained for various intra-vaginal tea tree oil based products. The MIC and MBC for 60 isolates of methicillin-resistant S. aureus (MRSA) and mupirocin-resistant S. aureus were 0.25% and 0.50%, respectively (Carson et al., 1995a). Carson et al., 1995b reported MIC90 of tea tree oil of 0.25% for *E. coli* and 0.50% for *S. aureus*.

*Thymus vulgaris* essential oil has very strong antimicrobial potential and depending on the bacterial strain tested it showed 3–8 times higher inhibitory activity compared with all tested antibiotics. It exhibited the same strength of antifungal activity as amfotericin and miconazol against *C. albicans* (Herman and Mlynarczyk, 2014). The thyme essential oil showed excellent inhibitory effect against both gram-positive and gram-negative bacteria especially against *E. coli* (Marino et al.,1999.) The essential oils of various *Thymus* species exibited strong antibacterial activity against Gram positive bacteria (*S. aureus*, *S. pyogenes* and *S. pneumoniae*) with the growth inhibition zone ranging from 10-54 mm for 25% dilution (Kulenova et al., 2000). *Thymus vulgaris* essential oil were found active against 30 strains of clinical isolates of *E. coli* including multi drug resistant strains (Sienkiewicz et al., 2011). It also exibited strong antibacterial activity against *S. aureus*, *B. cereus* and *P. vulgaris* (Al-Bayati, 2008).

*Origanum vulgare* showed a significant antimicrobial activity against various strain of *E. coli*, *S. aureus* and *Y. enterocolitica* (Sagdic, 2003). Strong antibacterial activity was also found against *Clostridium* genus which was demonstrated in up to 93% growth inhibition after 30 minutes of the exposure (Kačániová et al., 2014). The essential oils of *Origanum vulgare* obtained from the plants at different phenological stages showed antibacterial activity against both Gram-positive and Gram-negative bacteria with inhibition zones ranging from 9 to 36 mm (Béjaoui et al., 2013). Excellent antimicrobial activity was also obtained on multiple resistant strains such as *Stenotrophomonas maltophilia* MU 64, *S. maltophilia* MU 99, and *Chryseomonas luteola* MU 65 (Sarac and Ugur, 2008). *Origanum vulgare* were active against gram-positive bacteria (*S. aureus* and *B. subtilis*), gram-negative bacteria (*E. coli* and *P. aeruginosa*), a yeast (*C. albicans*) (Santoyo et al., 2006).

*Cymbopogon martinii* essential oil exibited very good antimicrobial activity against broad range of the microorganisms (Tsai et al., 2010) and reduced pro-inflammatory cytokines secretion in vitro. A strong antibacterial activity was also obtained against various strains of *E. coli* (Duarte et al., 2007).

*Pelargonium graveolens* essential oil exibited promising antimicrobial activity against broad range of microorganisms with inhibition zones ranging from 12 to 34 mm and MICs values from 0.039 to 10 mg/mL (Ben Hsouna and Hamdi, 2012). It exibited strong antibacterial activity against *S. aureus* which was comparble or even better compared to chloramphenicol and amoxicillin (Ghannadi et al., 2012). Strong sinergistic activity of *Pelargonium graveolens* essential oil and ciprofloxacin was observed against *K. pneumoniae*, *P. mirabilis* and *S. aureus* uropathogens (Malik et al., 2011).

## MATERIALS AND METHODS

### Study Design

673 patients in the age range from 19 to 65 years were subjected to Pap test, cervical swabs for the presence of anerobic bacteria, yeasts, *Ureaplasma urealyticum, Chlamydia trachomatis, Mycoplasma,* and hrHPV DNA. Among them 191 were positive to *Ureaplasma urealyticum* and represented our target group while the other patients were withdrawn from the study and subjected to conventional treatment depending on the diagnosis. Among 191 patients 94 was randomly selected to the experimental group and treated with doxycyclin (12 days) and herbal pessaries (10 days). The rest of 97 was selected into control group and treated with doxycyclin (12 days) and gyno-daktarin pessaries (10 days). Prior to the treatment all the patients signed the informed consent. To avoid possible intergroup differences the patients in both groups were of similar age and health status. Following the therapy all the patients were subjected to the Pap test and cervical swabs in order to determine the efficiency of the therapy. The sample preparation and analysis was described in details in our previous work (Findri-Guštek et al., 2012).

### Preparation of the Pessaries

The production of the macerate and formulation of the pessaries were described in details in our previous paper (Orescanin et al., 2015). The pessaries were composed of the following ingredients: *Calendula officinalis*, *Matricaria chamomilla*, *Lavandula officinalis*, *Hypericum perforatum* L., *Achilea millefolium*, *Thymus serpyllum*, *Salvia officinalis*, *Mentha piperita* L., *Symphytum officinale*, *Plantago major* L., *Alchemilla vulgaris*, *Olea europaea*, all macerated in olive oil; essential oils of *Melaleuca alternifolia*, *Thymus vulgaris*, *Pelargonium graveolens*, *Cympobogon martinii*, and *Origanum vulgare* L., Cera alba and Witepsol E75

### Statistical Analysis

For statistical evaluation Statistica 7.0 software package was employed. Statistical significance was set to $p<0.05$ in all the tests performed. The differences in the percentage of each parameter between control and experimental

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group, prior and after the therapy were assessed by $c^2$ test. The influence of the predictor variables (age, number of births, number of miscarriages, menopause, other health problems, Pap test, cervical swabs, type of therapy) on the response to the therapy was tested by Multiple regression method, General regression model and Canonical correlation analysis (Findri-Guštek et al., 2012; Oreščanin et al., 2015).

## RESULTS AND DISCUSSION

### Description of the Population

The population of the experimental group ranged from 19 to 64 years (34.4±9.3) with the highest percentage of woman (46.8%) ranging from 31 to 40 years. Woman ranging from 19 to 30 years represented 35.1% of the population while the rest of 18.1% of them belong to the group ranging from 41 to 64 years. The control ranged from 22 to 60 years (32.7±6.3). The highest percentage of them belongs to the age group ranging from 31 to 40 years (44.3%) and the lowest to the oldest group (17.5%).

The number of births varied from 0 to 4 in both groups. 58.5% of the experimental population and 52.1% of the control population had no childbirth. Among those who gave birth, women with 2 childbirths predominate (16% of experimental and 18.2% of control population). 98.9% and 97.9% of the experimental and control population respectively had no abortions, while 92.6% and 94.8% of experimental and control population, respectively had no miscarriages.

Menopausal women represent 7.4 % of the experimental and 4.1% of the control population.

Various health problems including allergies, hearth condition, high blood pressure, diabetes, hypothyroidism, benign tumors of the breasts and ovaries, malign tumors, depression, kidney dysfunction, Crohn's disease, genital herpes, skin conditions, stress were obtained in 22.9% of the experimental and 23.7 % of the control population. When distributed according to the age of the patients the health problems increased with age in both groups. Malign and benign tumors of the breasts and ovaries, allergies, hypothyroidism, skin conditions, stress, Crohn's disease, genital herpes and depressions prevailed in the patients younger that 40 while the patients ranging from 41-64 years suffered mostly from high blood pressure, diabetes, hearth and kidney conditions, equally in experimental and control group.

The results of Pap test showed no changes of the epithelial linings of the cervix in 23.4% of the experimental and 27.8% of the control population (Table 1). In both population low grade lesions prevailed while CIN II was observed in only 2.1% and 3.1% of experimental and control population, respectively.

46.8% and 46.4% of the experimental and control population, respectively was free from yeast, bacterial and viral infection with the exception of *U. urealyticum* which was isolated in all tested samples (Table 2).

Among 673 patients reported to the clinic, 191 (28.3%) of them were infected by *U. urealyticum*. Relatively high incidence was also reported by other researchers. Findri Guštek et al., 2012 found that 31.1% of the population was tested positive to *U. urealyticum*. was reported by. Young et al. 1981 found 69% and Pordeli et al., 2008 22.33% of the tested population positive to *U. urealyticum*. All the researchers found higher incidence among young, sexually active woman below 40 years of age.

22.3% and 19.6% of the experimental and control population was found positive to hrHPV. Among them the highest percentage in both groups (39.4% and 40.5% for experimental and control, respectively) was observed in the youngest age group (woman below 30). Those findings were in agreement with our previous studies (Findri Gustek et al., 2012; 2013) confirming the highest prevalence of hrHPV in young, fertile woman in the age group from 18 to 30. Similar findings have been reported by other researchers. Del Prete et al., 2008 reported the highest percentage (32.6%) of the HPV infected woman ranging from 20 to 30 years of age. Women ranging from 25 to 34 years of age showed the highest prevalence of HPV (24.2%) (Bardin et al., 2008). The incidence of HPV infection was inversely correlated with age (Bell et al., 2007). In women below 24 years HPV infection was significantly higher (41%, $p < 0.005$) compared to older age groups.

Among anaerobic bacteria *Gardnerella vaginalis*, a common cause of bacterial vaginosis predominated. It was isolated in 14.9 % and 17.5% of exposed and control population, respectively with the highest percentage in the youngest age group in both control and experimental popunation.

*Mycoplasma hominis* was isolated in 4.3% and 4.1% of experimental and control population. All the samples were isolated from woman below 30 years of age.

Similar results were also obtained for *Chlamydia trachomatis* with the prevalence of 3.3% and 4.1% in the experimental and control group. 4.8% prevalence of *C. trachomatis* was reported by O'Connell et al., 2009 while Colliers et al., 2009 found 2.9% samples positive to *C. trachomatis*. Findri Gustek et al., 2012. reported 2.5% of the tested population positive to *C. trachomatis*. All the positive samples were isolated from young, sexually active woman below 37 years of age.

According to the results of $c^2$ test there was no significant difference in the percentages between the control and experimental population for neither of the predictor variables or diagnosis.

### The Outcome of the Therapy

Following the therapy (Table 3) with doxycycline and herbal pessaries 91.5% of the patients were negative to *U. urealyticum*. Significantly lower percentage ($c^2 = 11.2$; $P = 0.0008$) was obtained for control group treated with doxycycline and gyno-daktarin pessaries (69.1%).

**Table 1. The results of Pap test for experimental and control group prior to the therapy**

| Diagnosis | Frequencies/percentages | |
|---|---|---|
| | Experimental | Control |
| NO | 22 (23.4%) | 27 (27.8%) |
| Inflammation | 2 (2.1%) | 3 (3.1%) |
| Mixed flora | 3 (3.3%) | 1 (1%) |
| Metalpasia | 5 (5.3%) | 9 (9.3%) |
| ASCUS | 17 (18.1%) | 15 (15.5%) |
| ASCUS/CIN I | 3 (3.3%) | 2 (2.1%) |
| CIN I | 40 (42.6%) | 37 (38.1%) |
| CIN II | 2 (2.1%) | 3 (3.1%) |

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**Table 2. The results of cervical swabs for experimental and control group prior to the therapy**

| Diagnosis | Experimental | Control |
|---|---|---|
| NO | 44 (46.8%) | 45 (46.4) |
| *Beta Hemolytic Streptococcus* | 2 (2.1%) | 0 (0%) |
| Chlamydia trachomatis | 3 (3.3%) | 4 (4.1%) |
| *hrHPV* | 21 (22.3%) | 19 (19.6%) |
| *Mycoplasma hominis* | 4 (4.3%) | 4 (4.1%) |
| *Candida albicans* | 4 (4.3%) | 5 (5.2%) |
| *Gardnerella vaginalis* | 14 (14.9%) | 17 (17.5%) |
| *Escherichia coli* | 1 (1.1%) | 3 (3.1%) |
| *Proteus mirabilis* | 1 (1.1%) | 0 (0%) |

**Table 3. The results of cervical swabs to *U. urealyticum* for experimental and control group following the therapy**

| Diagnosis | Experimental | Control |
|---|---|---|
| *NO* | 86 (91.5%) | 67 (69.1%)* |
| *U. urealyticum* | 8 (8.5%) | 30 (30.9%)* |

A significant difference ($c^2$ = 9.0; P = 0.0026) was also observed between experimental and control group in the treatment of other bacterial/yeast/viral infections (Table 4). 74.2% and 44.3% of the patients were free from the infection in experimental and control group, respectively. In the experimental group applied combined therapy completely eradicated *M. hominis, C. trachomatis*. It was also active against anaerobic bacteria with treatment efficiency of *G. vaginalis* of 85.7% and *P. mirabilis* of 100% that could be explained by synergistic activity of doxycycline and the ingredients of the pessaries with well proven antibacterial activity. New herbal pessaries showed strong anticandidial activity since none of the tested samples was positive to *Candida* species following the therapy. Some antiviral activity of the pessaries was also observed. Following the therapy 19% of the patients were negative to hrHPV. There was no improvement in the treatment of *E. coli* and *Beta Hemolytic Streptococcus* following the therapy.

Significantly lower efficiency was observed in the control group. There was no improvement in the treatment of hrHPV and *E. coli* while the efficiencies for *C. trachomatis*, *M. hominis*, *G. vaginalis* were 50%, 75% and 70.6%, respectively. Among the patients treated with miconazole based pessaries, 26.8% of the them developed antibiotic-associated yeast infection.

A significant improvement ($c^2$ = 23.3; P < 0.0000) was also observed in the results of Pap test following the doxycycline/herbal pessaries treatment with 80.9% of the patients with normal Pap smear (Table 5) compared to 23.4% prior to the therapy. Excellent wound healing potential of the extracts of medicinal plants used in the formulation of the pessaries could be responsible for such results (Oreščanin et al., 2015). 95% efficiency in CIN I eradication was observed. On the contrary, in the control group there was no significant improvement in the results of Pap test following the therapy ($c^2$ = 0.07; P < 0.9326).

**Table 4. The results of cervical swabs to bacteria, yeasts and hrHPV for experimental and control group following the therapy**

| Diagnosis | Experimental | Control |
|---|---|---|
| NO | 72 (74.2%) | 41 (44.3%)* |
| Beta Hemolytic Streptococcus | 2 (2.2%) | 0 (0%) |
| C. trachomatis | 0 (0%) | 2 (2.1%) |
| hrHPV | 17 (18.1%) | 19 (19.6%) |
| M. hominis | 0 (0%) | 1 (1%) |
| C. albicans | 0 (0%) | 26 (26.8%) |
| G. vaginalis | 2 (2.2%) | 5 (5.2%) |
| E. coli | 1 (1.1%) | 3 (3.1%) |

**Table 5. The results of Pap test for experimental and control group following the therapy**

| Diagnosis | Experimental | Control |
|---|---|---|
| NO | 76 (80.9%) | 30 (30.3%)* |
| Mixed flora | 2 (2.1%) | 2 (2.1%) |
| Metalpasia | 7 (7.4%) | 11 (11.3%) |
| ASCUS | 5 (5.3%) | 17 (17.5%) |
| CIN I | 2 (2.1%) | 34 (35.5%)* |
| CIN II | 0 (0%) | 3 (3.1%) |

When whole population was combined and subjected to Canonical analysis, Multiple regression analysis and General regression model obtained results leaded to the conclusion that selected variables had very good, statistically significant contribution to the outcome of the therapy (R=0.87; p<0.0000). However, according to the results of Multiple regression analysis and General regression model the type of the applied therapy was the only variable with statistically significant contribution (p<0.0000) to the outcome of the therapy (Fig. 1).

Possible difference in the results of two therapeutical approaches may lie in the fact that herbal pessaries are composed of the extracts and essential oils of the plants with well-known anti-inflammatory, wound healing, antimicrobial and antiviral effects. Consequently, they acts by reducing inflammation, induce wound healing, promote re-epithelization of the normal cells, prevent potential growth of microorganisms and act synergistically with doxycycline.

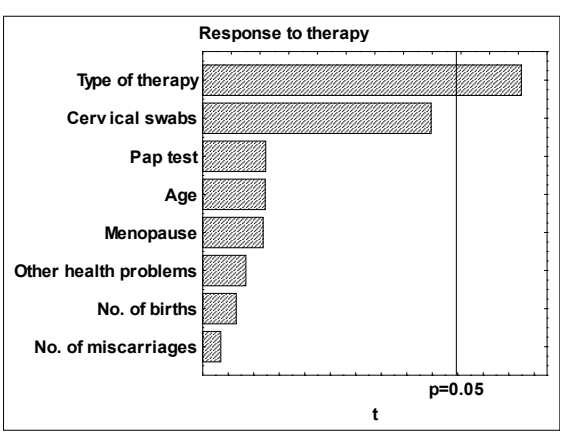


**Figure 1. Pareto charts of t-values for the influence of predictor variables on the response to the therapy**

## CONCLUSIONS

Considering the obtained results it could be concluded that new herbal pessaries based on the combination of macerates and essential oils of the selected medicinal

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plants could be used successfully for the prevention of antibiotic-associated yeast infections with superior efficiency compared to miconazole based pessaries. Preliminary results also showed their positive effect on the treatment of low grade CIN lesions. The synergistic effect of the medicinal plants with well known wound healing, anti-inflammatory, antimicrobial and antiviral properties could be responsible for high treatment potential of new herbal pessaries.

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