

Pharmacological, Microbiological and Clinical Activity of Feminine Intimate Cleansers Based on Plant Extracts Active Principles (Saugella Line)

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Abstract

Introduction: The use in intimate hygiene of active substances plays an important role in maintaining the balance of the vaginal ecosystem. The most medically based cleansers are promoted to doctors through a classic medical information, even if classified as cosmetics.

The present paper assessed the reliability of the pharmacological, microbiological and clinical claims both on the active principles of *Salvia officinalis*, *Thymus vulgaris*, *Camomilla recutita* and *Calendula officinalis* extracts, contained in the most used hygiene product line.

Methods: The experimental activity was studied on the inhibition of microbes' adhesion to human vaginal epithelial cells, morphostructural damages to germ cells, inhibition of microbial biofilms, and anti-inflammatory tests.

The clinical data were collected according to the use recommendation as daily intimate cleanser, uid or bid for four weeks: salvia (SD - Saugella Dermoliquido) in fertile age women, thymus (SA – S. Attiva) in pregnancy, breastfeeding and risk of vulvovaginal infection, chamomile (SP – S. Polign) in postmenopausal women, calendula (SG – S. Girl) in pre-adolescent girls prone to vaginal irritations. At baseline and after 4-week treatment, vaginal pH, signs/symptoms, and sexual activity were evaluated.

Results: The experimental data showed the antibacterial, antimycotic, anti-inflammatory and antioxidant activity of the natural principles contained in the four extracts.

The clinical studies evidenced the significant reduction of genital signs and symptoms (itching, burning, erythema, edema, vaginal dryness, dyspareunia) and the improvement of sexual female disorder.

Conclusions: Correct intimate hygiene improves quality of woman's life and can prevent minor vulvo-vaginal pathologies. The cleanser selection should be tailored on the age and physiopathological condition of the woman.

The claims of efficacy and safety that supports the recommendation of the use in daily practice of the Saugella line products containing Salvia, Thymus, Chamomile, and Calendula extracts are consistent and reliable.

Keywords: Vaginal pH; Candida; Bacterial vaginosis; Intimate hygiene; Plant extracts; Sexual activity

Introduction

Vaginal ecosystem is an ecologic niche in which microorganisms, physical characteristics (pH, mucus viscosity, etc.) and biochemical processes are in balance.

The vaginal environment often contains microorganisms that become pathogens only due to changes in the ecosystem favored by the anatomical proximity to the rectum and bladder, organs that can contaminate the vaginal habitat. Among these, the most represented germs are Candida, Mycoplasma and Gardnerella vaginalis. The use in intimate hygiene of active substances plays an important role in maintaining the balance of the vaginal ecosystem in order to prevent overt pathological conditions.

An adequate protection of genital area plays an important role in keeping vaginal ecosystem in good balance, naturally protected by Döderlein lactobacillus, producing lactic acid which keeps vaginal pH below 4.5, and by natural chemical, physical and anatomical mechanisms of defense of vulvo-vaginal passage.

The optimal condition of vaginal ecosystem favor a better quality of life, confirmed by the strict relationship among correct intimate hygiene, good vulvovaginal state, physiological vaginal pH and better sexual satisfaction [1].

The first step for clear improvement of intimate health is to adopt a correct life style, that include diet (a higher intake of sugars

corresponds to a higher yeast formation) sexual activity, clothing and correct procedure of vulvo-perineal hygiene.

Intimate wellness is at risk due to the daily use of synthetic underwear, panty liners, sanitary towels, tight trousers, tights, that creates a barrier effect, impedes transpiration and increases the temperature, reproducing the conditions of a "vulvar occlusive bandage" or "greenhouse effect". As a matter of fact, the lack of ventilation, the obstacle to transpiration, a warm humid environment, vaginal secretion rich in germs and micro traumas due to the friction of clothing, create a favorable soil for microbial growth at level of vulvar vestibule. These conditions stimulate the proliferation of germs, and are often associated to a higher presence of vaginal candidiasis or bacterial vaginosis [1].

On the other hand, the use of broad-spectrum antibiotics can reduce lactobacilli production and diminish the natural defenses that in

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Received June 08, 2015; Accepted July 06, 2015; Published July 08, 2015

Citation: Leo VD, Benvenuti C (2015) Pharmacological, Microbiological and Clinical Activity of Feminine Intimate Cleansers Based on Plant Extracts Active Principles (Saugella Line). J Women's Health Care 4: 244. doi:10.4172/2167-0420.1000244

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the long term could be counterproductive: eg by reducing the frequency of successful *in vitro* fertilization [1].

The second step for maintenance of good health of the genital area is represented by a correct intimate hygiene, aimed to protect from microbial and physico-chemical attacks. This procedure is very important for both the ancillary treatment and, mainly, the prevention of only apparently minor vulvovaginal diseases. Inappropriate hygiene during the month and inadequate menstrual protection can contribute to the onset and/or maintenance of vulvovaginal troubles. On the other hand, too frequent genital washing, more than 2 to 3 times a day, should be avoided, as it removes the layer of sebum that is essential to protect the mucosa.

The use of over-aggressive detergents or detergents with an inadequate (neutral or alkaline) pH exposes it to inflammatory processes caused by physical (microtraumas), chemical (soaps, allergens) and infective attacks (bacterial, mycotic, parasitic and viral).

The remarkable progress in cosmetological Research & Development in acquiring innovative substances, widen the target of the cleanser use and give clinical answer to more specific populations, as pre-pubertal girls, fertile age women during menstrual cycle, pregnancy and post-partum, situations at risk of microbial infections, menopause and women with tendency to genital area dryness because of sensitivity to normal cleansers.

Intimate hygiene is a simple routine activity, but the differences related to the age and physiopathological condition of the woman, which influence the balance of vaginal ecosystem, require choosing the appropriate formulation for each individual.

Phytotherapy anticipated modern pharmacotherapy and still now find a rational role in many clinical conditions of mild to moderate severity, sparing treatments that are more aggressive. Officinalis plants are known to provide active principles, useful for pharmaceutical preparations of standardized high quality. More recently, applied research could document the pharmacological, microbiological and clinical activity of these active principles, whose historical use was based on the empiric popular tradition.

A number of plant extracts underwent an accurate pharmacological, microbiological, technical and clinical screening aimed to choose the most suitable natural active ingredients for the applicability in gynecology. The most used plant extracts in intimate hygiene formulations are: *Salvia officinalis* extract, containing active principles such as salviol, pricosalvin and pinene, responsible for the antibacterial, antimycotic, antioxidant, and antiviral activities; *Thymus vulgaris* extract containing thymol and carvacrol, active phenolic derivatives endowed with a marked and selective antibacterial, antimycotic, antioxidant and anti-inflammatory activity; *Chamomilla recutita* extract containing the cyclic alcohol α -bisabolol with gives anti-inflammatory, lenitive and decongestant action and *Calendula officinalis* extract for its itching lenitive, anti-inflammatory, antiedema, antiviral, and antioxidant activity.

Products for intimate hygiene are classified as Cosmetics, i.e. a category of products that only recently were ruled by Health Authorities that approved guidelines for the study, safety and claim of cosmetics. In practice, claims are allowed only if supported by scientific data and a structured system of cosmetovigilance is compulsory [1].

The present paper analyzes the pharmacological, microbiological and the available clinical documentation both on the active principles contained in *Salvia officinalis*, *Thymus vulgaris*, *Camomilla recutita*

and *Calendula officinalis* extracts and in the final product in toto, with the aim to verify the reliability of the scientific claims on activity and safety of this product line, amounting for more than half of the existing products of its class.

Methods

The evaluation of the effects of the active principles contained in the extracts and of the cleansing product in toto as used in practice, is based on the experimental and clinical data published in international journals or presented at scientific congresses as posters and asked to the manufacturer.

Assessment of pharmacological and microbiological activity

The following methods were used:

- Antibacterial and antimycotic evaluation by the agar diffusion method.
- Inhibition of microbes' adhesion to human vaginal epithelial cells (VECs), to assess whether thymol, the major component of thyme oil, interferes with the adhesion of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Gardnerella vaginalis* to VECs. Microbial adhesion was determined by the ratio $\frac{\text{number of adhering microbial cells}}{100 \text{ VECs}}$ in a scanning electron microscope (SEM).
- Morphostructural damages to *C. albicans* envelopes induced by thymol, and examined through SEM and atomic force microscopy investigation (AFM).
- Inhibitory activity of thymol against the formation and viability of *C. albicans* hyphae, that are an important virulence factor in the pathogenesis of vaginal candidiasis. The counting of the differentiated forms and spherical cells was performed in Burkert's-chamber under fluorescence microscopy.
- In vitro* inhibition of native and mature *C. albicans* and *G. vaginalis* biofilms induced by thymol by means of spectrophotometric analysis, Nomarski interference contrast microscopy, and fluorescence microscopy with live-dead cell visualization.
- Antioxidant potential investigated through the Luminol Amplified Chemiluminescence (LACL) test. Anti-inflammatory activity, measuring the ability of a substance to antagonize the release of human neutrophil elastase, a recognized inflammation model.

The microbiological inhibition tests were investigated at 1MIC (125 $\mu\text{g/ml}$), and serial dilution up to 1/64 MIC of thymol.

Clinical studies

The clinical data were collected according to an open, observational, perspective design for each extract corresponding to a specific cleanser: namely *Salvia officinalis* (SD - Saugella Dermoliquido), *Thymus vulgaris* (SA - Saugella Attiva, in which thyme and sage extracts are combined), *Camomilla recutita* (SP - Saugella Poligyn), *Calendula officinalis* (SG - Saugella Girl) all manufactured by Rottapharm SpA, a MEDA company).

The women were stratified into different subgroups, (adolescent, fertile, pregnancy, breastfeeding, and menopause) and the most appropriate detergent was recommended, on the basis of her age and physio-pathological status: SD in adolescent, fertile, and pre-

menopause; SP in menopause; SA in pregnancy, breastfeeding and in presence or risk of vulvovaginal infection and SG in pre-pubertal girls. Diagnosis of bacterial vaginosis and candidosis was mainly based on clinical examination as routinely done in daily practice, with the addition of a microbiological evaluation in about half of the cases.

Each cleanser was used once or twice a day for four weeks.

At baseline and after 4-week treatment, vaginal pH, signs/symptoms, sexual activity (score=absent 0, poor 1, good 2, very good 3) any clinical condition and ongoing pharmacological treatments were assessed. Vaginal pH was measured using a standardized kit distributed to all of the gynecologists at the start of the study. The data were statistically analyzed using Student's t test or the χ^2 test as appropriate.

Clinical pharmacology study in fingertip dipping method

Transient micro-organisms, which are acquired as a result of contacts with contaminated objects and the environment, colonize the surface layers of the skin and are less adherent. They can be transferred during contacts with people or contaminated environmental surfaces, and are more easily removed by hand washing. The role played by hands in spreading microorganisms and infections is recognized throughout the world, and hand washing is important as a means of reducing contamination of transient micro-organisms.

This study evaluated the antimicrobial activity of detergent SA on hand surface micro-flora using the fingertip dipping method.

Results

The pharmacological and microbiological properties of the active principles contained in the examined plant extracts.

Sage extract

The active principles contained in the sage extract showed bactericidal activity on gram-negative and -positive bacteria at dilutions of 1/4000 and bacteriostatic activity at dilutions of 1/10000, more marked on gram-positive organisms [2-7].

The microbiological and anti-inflammatory spectrum of sage extract is completed by antagonistic effect against fungi, viruses, free radicals and vascular permeability [8-14].

Clinical activity of *Saugella dermoliquido* in feminine intimate hygiene

The pivotal clinical data available on the cleanser containing sage extract at pH 3.5, *Saugella Dermoliquido*, were collected in 1354 women: 241 adolescents (17.2 ± 1.7 yrs) (mean ± SD), 861 fertile (32.1 ± 8.0), 252 pre-menopause (47.6 ± 4.4).

Mean vaginal pH was significantly reduced to normal values after four-week use of SD in all groups: in adolescents from 4.96 ± 0.2 1 at baseline to 4.50 ± 0.16 end of treatment (p<0.01), in fertile women from 4.94 ± 0.15 to 4.56 ± 0.12 (p<0.01) and in pre-menopause from 4.99 ± 0.19 to 4.62 ± 0.17 (p<0.01). Female sexuality after daily use of SD for intimate hygiene significantly improved after 4 weeks application (Figure 1).

Thyme extract

Thymol and carvacrol, main active principles of extract of thyme, cause a break of the outer liposaccharidic layer of the cell membrane, followed by the partial disintegration of the external membrane [15]. Thymol and carvacrol have a synergic effect.

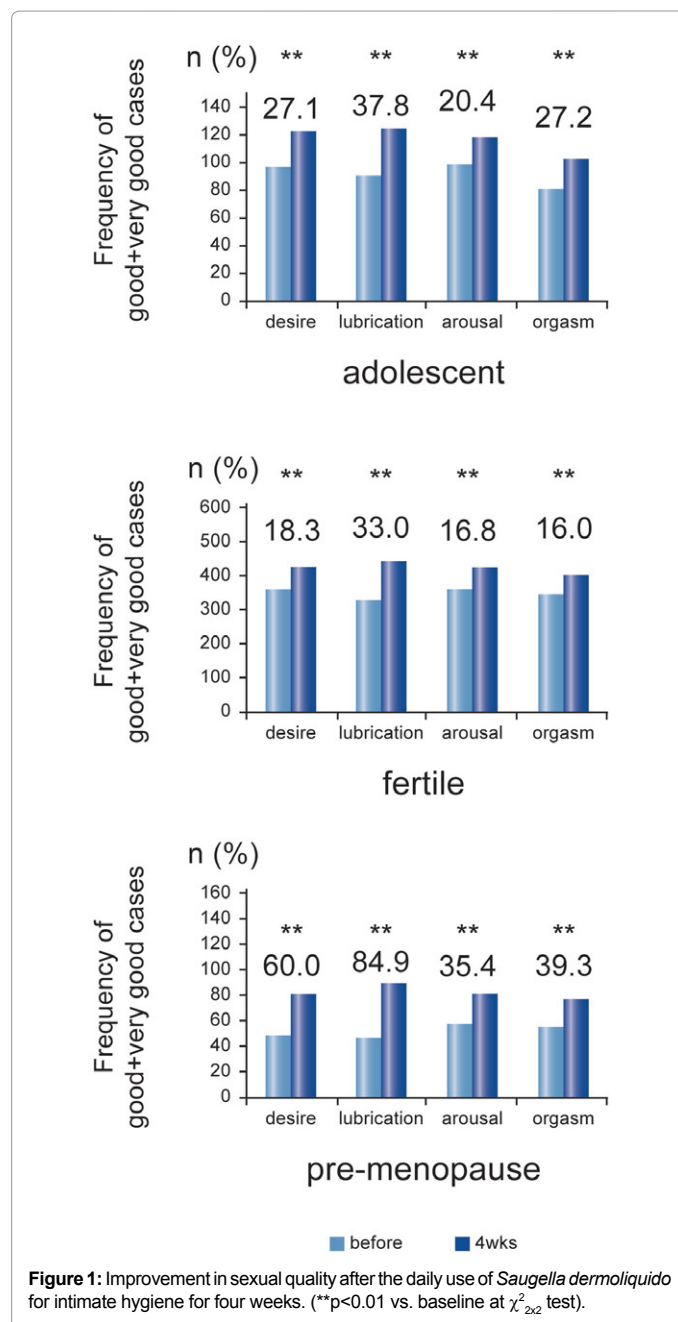


Figure 1: Improvement in sexual quality after the daily use of *Saugella dermoliquido* for intimate hygiene for four weeks. (**p<0.01 vs. baseline at χ^2_{2x2} test).

In particular, the thyme extract showed antibacterial activity on *G. positives* and *G.negatives*, leaving *Lactobacilli* unchanged with a selective action and antimycotic activity [16-20]. It reduces microbial adhesiveness of *E. coli* (up to 1/32 MIC), *S. aureus* (up to 1/16 MIC), *G. vaginalis* (up to 1/16 MIC) and *C. albicans* (up to 1/8 MIC) to human vaginal cells at doses below the MIC (minimum inhibitory concentration); it induces morpho-structural damages of *Candida* envelope; it interferes with the *C. albicans* filamentation; it has antioxidant and antiinflammatory actions [19,21-29].

The AFM micrographs showed striking morpho-structural deformities such as flattened cells with surface folds, cells with holes, collapsed cells and ghosts, which become more numerous at increasing concentrations and times of incubation [30].

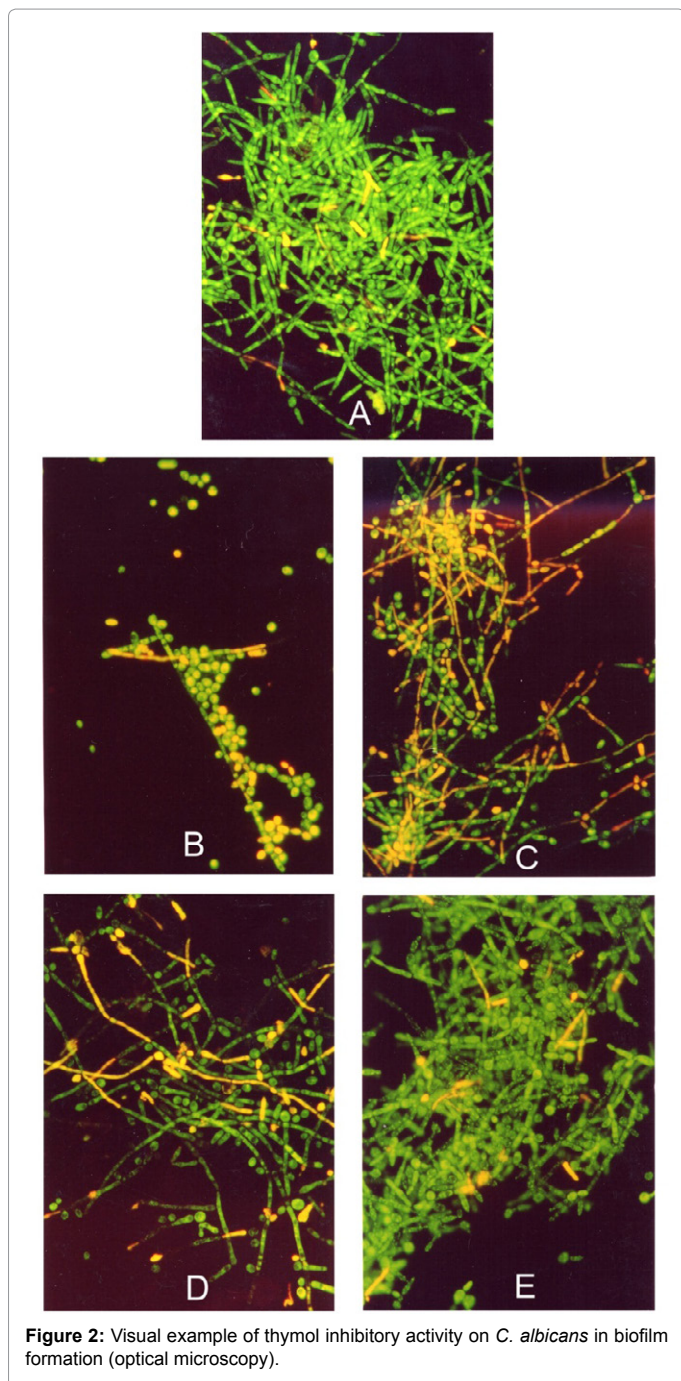


Figure 2: Visual example of thymol inhibitory activity on *C. albicans* in biofilm formation (optical microscopy).

Thymol inhibits native and mature biofilms of *Candida* from 1 MIC to 1/8 MIC and *Gardnerella* from 1 MIC to 1/4 MIC [31,32] (Figure 2).

Clinical activity of Saugella Attiva in feminine intimate hygiene

The clinical data on SA, cleanser containing thymus extract as main component at pH 3.5, were collected in 819 women: 484 in pregnancy mean aged \pm SD 30.5 yrs \pm 4.5, and 335 in puerperium, aged 31.9 yrs \pm 6.1.

Specific treatments used in concomitance with extract of SA were local antimycotics in 0.76% of cases and antiseptics in 0.25%; systemic antibiotic treatments were indicated in 0.13%.

After four-week' use of SA, mean vaginal pH reached normal values in pregnancy (from 4.92 ± 0.17 at baseline to 4.59 ± 0.13 end of treatment, $p < 0.01$ vs. baseline), and in post-partum (from 5.17 ± 0.20 to 4.64 ± 0.17 , $p < 0.01$).

Both vaginal candidiasis and bacterial vaginosis showed a significant reduction in pH to normal values (from 5.25 to 4.51 in bacterial vaginosis and from 5.29 to 4.41 in mixed forms with $p < 0.0001$ vs. baseline).

In vaginal candidiasis and bacterial vaginosis, the specific symptoms were consistently present at baseline: respectively itching 71.9% and 61.1%, burning 73.4% and 79.2%, edema 63.3% and 62.5%, erythema 59.4% and 63.9%. In pregnancy and post-partum, the presence of symptoms at start was lower, respectively itching 44.9% and 44.0%, burning 47.3% and 60.4%, edema 39.9% and 46.8%, erythema 35.3% and 45.4%.

In all groups, the symptoms disappeared in more than 60% of cases, with a highly statistical significance, and in the meantime leucorrhoea, vaginal micro-flora and female sexuality disturbances improved after four weeks' daily use of SA for intimate hygiene (Figure 3).

Clinical activity of thymol in hand washing

The simply dipping fingers in SA without rubbing, gave a significant reduction in bacterial counts in comparison with control after 4 (47.6%) and 8 minutes (61.9%) ($p < 0.01$) [33] (Figure 4).

Camomile extract

Alfa-bisabolol contained in chamomile essential oil, has antifungal, anti-inflammatory, antioxidant, antiedema and soothing properties [34-36].

Clinical activity of Saugella Poligyn in feminine intimate hygiene

The pivotal clinical data on the SP, cleanser containing chamomile extract combined with *Thymus vulgaris* extract as main components at pH 7.0, were collected in 418 women, men aged \pm SD 58.6 yrs \pm 7.5. In this population, the specific symptoms were consistently present at baseline: respectively itching 41.7%, burning 44.2%, oedema 80.9%, erythema 60.7%.

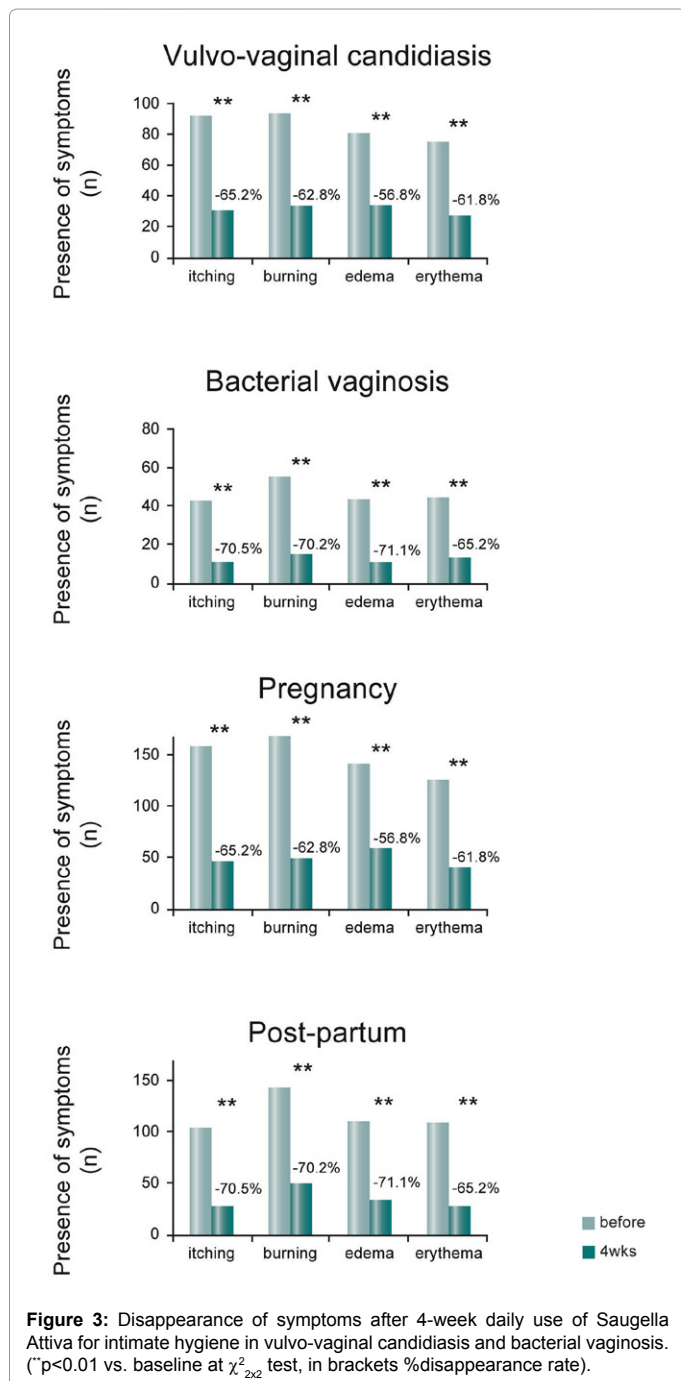
Four-week intimate hygiene with SP in menopause normalised mean vaginal pH from 5.14 ± 0.20 at baseline to 4.98 ± 0.17 and significantly reduced genital signs and symptoms ($p < 0.01$): in particular, vulvar itching disappeared in 47.5% of cases, burning in 47.3%, vaginal dryness in 24.7% and dyspareunia 27.0%. Moreover, a statistical significant improvement of sexual female disorder was observed after 4 weeks of daily use of SP (Figure 5).

Calendula extract

Calendula officinalis extract has anti-inflammatory, anti-edema, antiviral, antibacterial, antioxidant and healing activities and provides itching relief. It is known for its refreshing, restorative and protective due to the synergistic action of triterpene alcohols, flavonoids and polysaccharides content. It is particularly suitable for the care and restoration of dry skin, chapped skin, delicate and easily reddening. It carries protective activity against contact dermatitis [37-44].

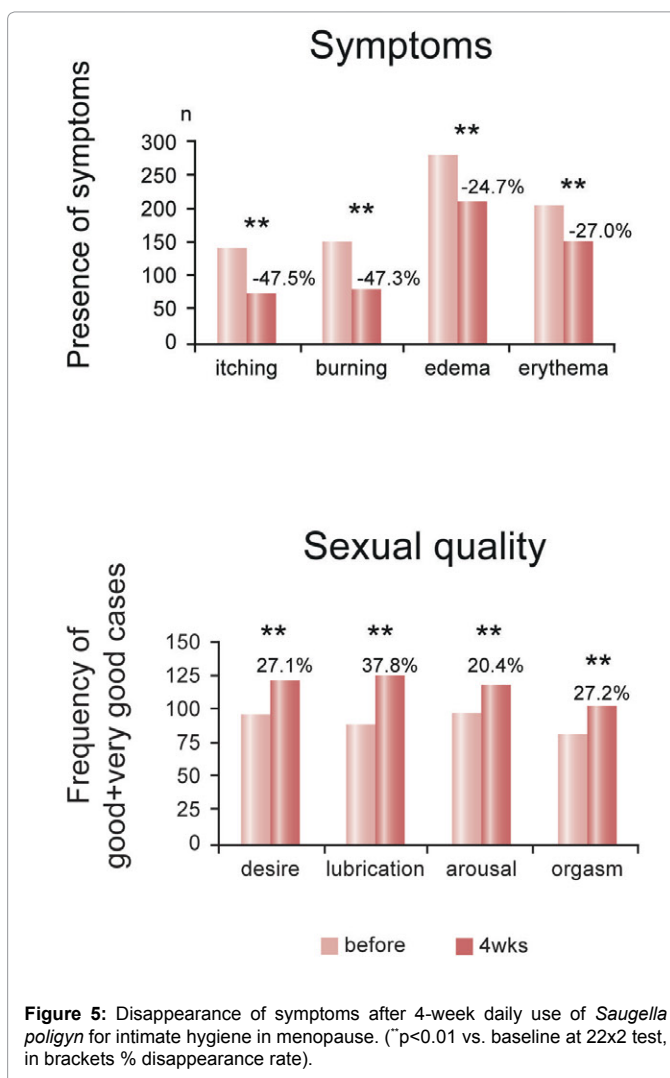
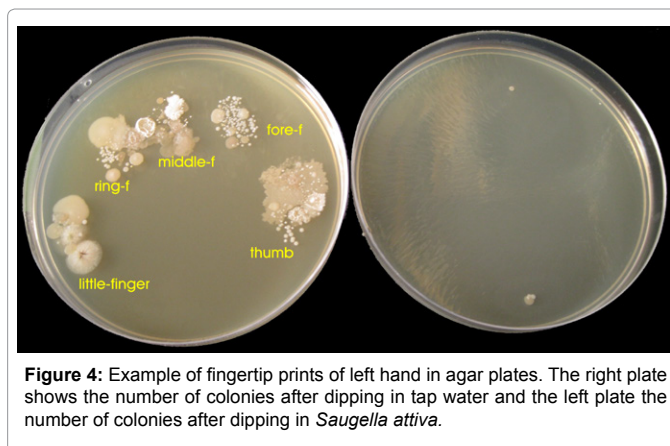
Clinical activity of Saugella Girl in feminine intimate hygiene

Calendula officinalis and *Malva sylvestris* extracts, endowed with anti-inflammatory, antibacterial, antimycotic and emollient



action, Avena sativa, that reduces itching through a hydrating and decongestant action, and rice proteins, which give elasticity to the tissue, were combined in detergent formulation at pH 4.5 (Saugella Girl). The clinical activity and the acceptability of SG were studied for a correct daily intimate hygiene in pre-pubertal children [45]. The sample observed was represented by 40 children suffering vulvovaginitis, mean aged 7.2 years \pm 2.5 (SD 3-12 min-max), in which 10 out of 40 presented a concomitant allergy. Itching, burning, vulvo-vaginal edema and erythema were progressively and clearly reduced during the treatment with SG (Figure 6).

At study start, the vaginal swab was positive in 7 case out of 20, with 9 bacterial strains (Streptococcus in 3 subjects and Haemophilus



in 5 subjects) subsequently disappeared during the 2nd week of SG. At that time, Streptococcus β -haemolyticus group F and H. parainfluenzae were detected respectively in two distinct subjects, both disappeared in the 4th week of product use. Vaginal discharge normalized since the 13th day. At the end of the study, the physician's judgment was good + very good in 94.8%. After SG use, 75% of the girls reported an

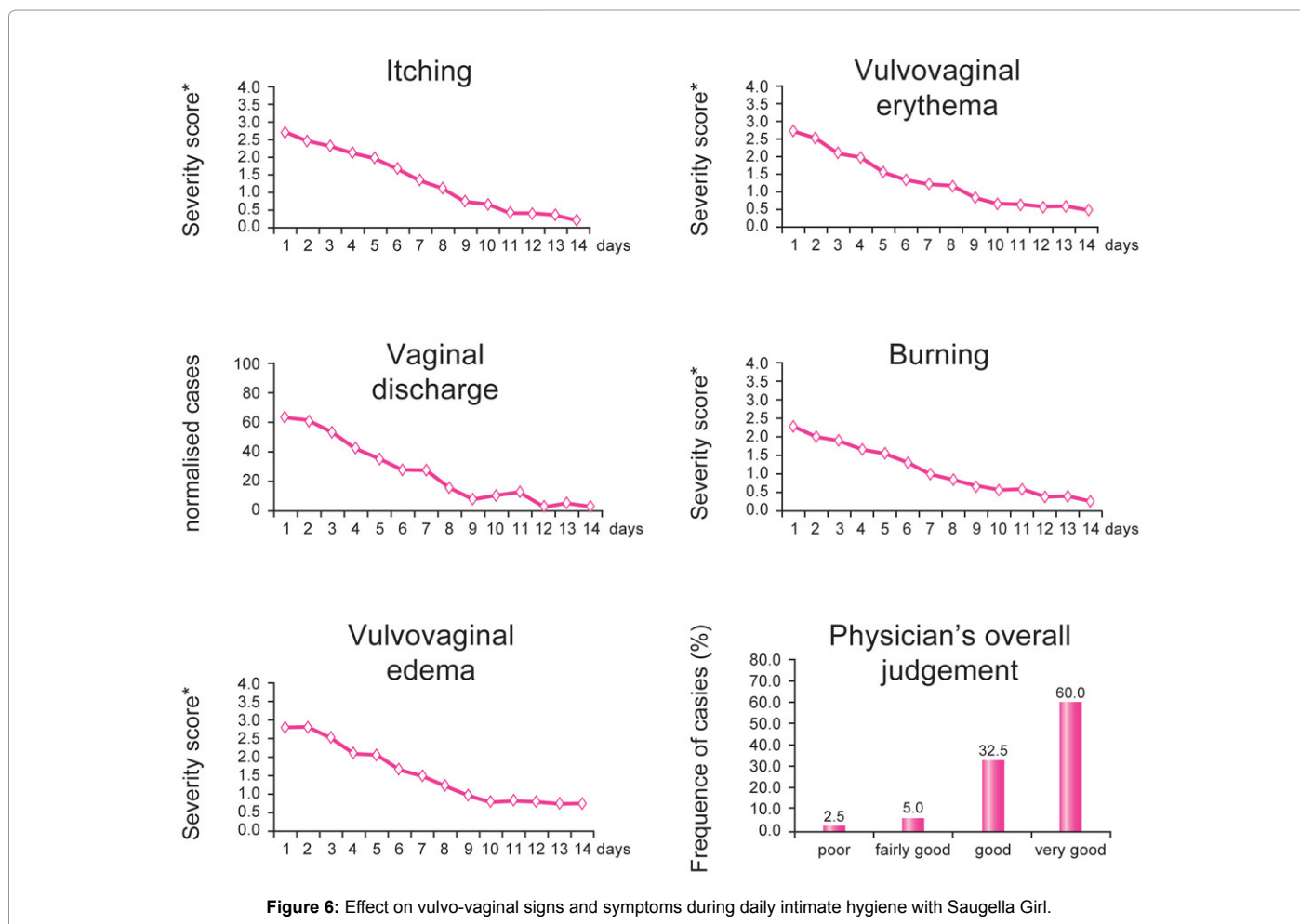


Figure 6: Effect on vulvo-vaginal signs and symptoms during daily intimate hygiene with Saugella Girl.

improvement of symptoms previously complained, while in 1 case only (2.5%) an impairment was reported.

Discussion

Correct intimate hygiene is a useful step to prevent minor vulvo-vaginal pathologies and more serious systemic consequences, often underestimating more serious consequences. A good parallelism is represented by hand washing, that, removing external transient microorganisms, prevents their transfer to susceptible subjects or inside the body.

Washing hands is an external action, which prevents internal diseases: prevention of infections is obtained in hospitals with accurate hand hygiene and the best evidence for the prevention of the common cold supports physical intervention e.g. hand washing. On the other hand the WHO's 2014 global annual campaign state: Clean Care is Safer Care, clean your hands and save lives [46,47].

The correctness of this rationale is confirmed by the clinical results here presented that showed how the use of natural substances in daily intimate hygiene can actively contribute to maintaining/restoring conditions of vulvo-vaginal integrity, leading to the reduction in symptoms and the stabilization of physiological vaginal pH values, improving the women intimate well-being. The rationale of the study is to underline the importance of practicing daily intimate hygiene for genital health of women, and to draw the attention to the activity of plant extracts.

This was the first systematic clinical review on an intimate hygiene product, and it was carried out on an observational basis. It can be considered a hypothesis generator study, hopefully followed by both microbiological and clinical controlled designs [48].

An appropriate intimate cleansing can be an important prevention tool in woman daily life, as shown by the significant relationship among correct hygiene, vulvovaginal health, optimal pH and better sexual satisfaction [49].

The here examined natural substances evidenced strong of pharmacological and clinical activity, as shown by many independent and international sources.

Maintaining a healthy vaginal ecosystem and recovering it from mild/moderate inflammatory/infectious conditions, concomitantly, if needed, with a pharmacological therapy, can prevent the complications of bacterial vaginosis that, even if it is not a life-threatening disease, is recognized as a contributory cause of premature membrane rupture, premature birth and low birth weight.

Saugella line products showed to be effective thanks to the content in natural active principles derived from plant extracts. Moreover, the acid pH of its formulations is always favourable in preventing or treating infections, even in mycoses as acid pH counteracts the very frequent bacterial superinfections [50].

In facts, the extracts of *Thymus vulgaris* and *Salvia officinalis* have

clear antibacterial and antimycotic activities, combined with scavenging action against reactive oxygen species, all important features for their use in daily practice [51]. The propaedeutic experimental activity of *Salvia officinalis* confirmed the suggestion of its Latin etymology (health or salvation), making it suitable for use in preparations intended for daily intimate hygiene in women of child-bearing age.

The maintenance of intact envelope architecture is an important factor in the virulence of fungi and thymol induces morpho-structural damages and affects the envelope of *Candida* as a monoterpene [26]. In fact, terpenes enter the fatty acyl chains of membrane lipid bilayers, disrupt lipid packaging, and alter membrane permeability. They also inhibit *Candida* respiration, thus suggesting adverse mitochondrial effects. All of these phenomena lead to major surface alterations and deformities that reduce the ability of the fungi to adhere to mucosal cells, thus decreasing their virulence and infectiousness and supporting the usefulness of thymol in the clinical management of candidiasis, particularly muco-cutaneous presentations such as vulvovaginal candidiasis.

The importance of germ tube/hyphae formation for the invasive capability of *C. albicans* has been stressed and these findings show that thymol is able to disturb cell membrane and *Candida* metabolism, probably by affecting also the fungal-cell-wall synthesizing enzymes [25].

Thymol can interfere with the mechanisms of bacterial and fungal adhesion to human vaginal epithelial cells at doses below the MIC (minimum inhibitory concentration) that allows to inactivate pathogens even at low concentrations. This is of interest for the strategy of protecting against vaginitis or vaginosis using compounds other than antibiotics and antimycotics [21-23].

Biofilms are known to be important factors underlying virulence and pathogenicity of germs, and both *C. albicans* and *G. vaginalis*, the predominant specie in bacterial vaginosis, have a high propensity to develop biofilms that allow microbes to tolerate higher concentrations of antibiotics, thus increasing the possibility of recurrence. The ability of thymol to antagonize native and mature biofilm of *Candida* and *Gardnerella* is an important feature.

In conclusions, the extract of *Thymus vulgaris* have properties that make it suitable for use in preparations intended for daily intimate feminine hygiene.

In pre-pubertal age, vulvo-vaginal ecosystem is more frequently liable to inflammations and infections than up to now thought. They are at relative risk of vulvovaginitis, due to anatomy, physiology, behaviour and environment. The predisposing factors to vaginal mucosa infections in prepubertal age are: a relatively exposed vulva, a thin vaginal wall, poor hygiene, heat, humidity, and clothing. Vaginal mucosa is thin, lack of cornification and has a basic pH which expose it to pathogen invasions. Asymptomatic children are positive for aerobes (77%), for anaerobes (65%) and for both (45%). The use in intimate hygiene in this range of age of a detergent based on natural extracts of *Calendula officinalis* and *Malva sylvestris*, as Saugella girl, showed to be appropriate both for the coadjuvant treatment and the protection of vulvo-vaginal tissues from inflammatory conditions of different origin. The product can exploit also healing properties as shown by the increase of the speed of episiotomy wound healing.

It is important to underline that the antibacterial and antimycotic activity of natural plant extracts does not induce any resistance, as proven by the fact that they are still currently active, notwithstanding

the use of these compounds was common since the ancient Greek and Romans times.

Hence, intimate hygiene should not be intended as an alternative treatment to pharmacological therapy in presence of clear infections, but a relevant action to be taken for completing, or preventing, antibiotic use. Following these considerations, the doctor's goal is to encourage hygiene practice contributing to reduce the occasions to turn to antibiotics when unnecessary, so minimizing resistance induction.

Conclusions

Correct intimate hygiene is a key point in woman daily life, for an adequate protection of genital area, preventing minor vulvo-vaginal pathologies

An appropriate cleansing should consider the differences related to the age and physiopathological condition of the woman, in choosing the most suitable formulation for each individual.

The claims concerning the here reviewed products containing plant extracts are consistent and reliable. *Salvia officinalis*, *Thymus vulgaris*, *Chamomilla recutita*, and *Calendula officinalis* of Saugella line, guarantees the presence of active principles endowed with antibacterial, antimycotic, antioxidant, and antiinflammatory activities and can be useful in preventing or restoring the balance of the vaginal ecosystem in fertile age (SD), pregnancy and breastfeeding (SA), postmenopausal women (SP), young and pre-adolescent girls (SG). As these substances are promoted to doctors through classic medical information, it is important doctors to know the scientific basis that supports the recommendation of their use in daily practice.

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