Original Article

A New Vaginal Gel for Femalegenital Wellness and Disease Prevention: Rationale of a Multi-Herbal Derived Treatment on a High-Tech Pharmaceutical Perspective

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ABSTRACT

The folk ethno-medicine describes some useful herbal remedies for vaginal hygiene, whose knowledge goes back to previous centuries; unfortunately, only few of them have been submitted to clinical trials and fulfilled the evidence-based medicine rules thus being successfully introduced into the routine use; as a matter of fact actually the consumer is quite sensitive to the message that historical natural active principles revisited by positive recent investigations can have a role in the disease prevention and treatment.

Salvia, *Melaleuca* and *Calendula* have been selected by us among a certain number of natural compounds to be introduced in the formula of our vaginal gel, because their complementary properties synergize each other, achieving, altogether the best clinical results. The mechanism of actionof the 3 herbs extracts in combination, takes control of the vaginal surface integrity and mucosa restoration after mechanical, chemical or microbiological damage, especially through the well-known *Calendula* contributes to body defenses against external agents, has anti-inflammatory, antioxidant and healing properties; *Salvia* expresses altogether a wide and selective control, supports the natural mechanism for body´s purification and promotes the toxin elimination; *Melaleuca* expresses altogether a wide and selective control of pathogenic microflora enclosing the often relapsing *Candida albicans* super-infections.

Keywords: Vaginal wellness, Sage, Marigold, Tea Tree oil, Vaginal gel.

INTRODUCTION

Vaginal Gel Based upon Herbal extracts and HPMC

"The vaginal gel" is a water-based vaginal gel with a lubricant action, thanks to the synergy of hydroxypropyl methylcellulose (HPMC) associated with distilled waters of *Salvia officinalis L.* and *Calendula officinalis L.*, useful to keep lubricated and protected the vaginal surface. The *Melaleuca alternifolia* (tea tree oil) with the other components, gives to the gel a soothing and refreshing properties. To the formula is added chlorhexidine, with antifungal and bacteriostatic properties just with preservative purpose to inhibit micotic and bacteria contamination in the preparation.

The natural herbal resources are very helpful in maintaining a well balanced environment when topically delivered in the mucosal cavities of the human body because of the multifactorial interferences of the phytocomplexes with the biological background. Specifically an healthy vaginal homeostasis has multi-steps rebounds, either in the endocrine and immunological setting, or in the mucosa cells turnover and trophic activation, in the maintenance of acidic pH, optimal hydration or finally in the symbiotic microscopic flora control. The folk ethno-medicine describes some useful herbal remedies for vaginal hygiene, whose knowledge goes back to previous centuries; unfortunately, only few of them have been submitted to clinical trials and fulfilled the evidence-based medicine rules thus being successfully introduced into the routine use; as a matter of fact actually the consumer is quite sensitive to the message that historical natural active principles revisited by positive recent investigations can have a role in the ailment prevention and treatment.

Salvia, *Melaleuca* and *Calendula* have been selected by us among a certain number of natural compounds to be introduced in the formula of our vaginal gel, because their complementary properties synergize each other, achieving, altogether the best clinical results. The dry powder of each plant extract was in amount of 100 mg and added to 500 ml of distilled water then boiled for one minute to obtain the aqueous extract, therefore the plant concentration is lower and it not explicates pharmacological action. Chlorhexidine has been added at the concentration of 0, 02%, this low concentration is addressed to prevent contamination of the gel and packaging by bacteria and protozoa. Hydroxypropyl methylcellulose, a polymer with muco-mimetic properties has been added to induce a protective, transparent and viscoelastic shield on the mucosa upon which the active principles can express their mixed properties at the best being. *Salvia* and *Calendula* in the soluble form of water extracts, *Melaleuca* (tea tree oil) as essential oil.

Individual clinical background for *Melaleuca*, *Salvia* and *Calendula* mixed formula

*Calendula officinalis Linn (*Marigold)

Calendulaofficinalis L. (*Asteraceae*) iscommonly used for quickening the wound healing process due to its anti-inflammatory, anti-virus, antimicrobial and antifungal activity, anti-cancer, antioxidant and healing properties[1]. The major classes of *Calendula* phytoconstituents are triterpene alcohols, triterpene saponins, flavonoids, carotenoids and polysaccharides. Some of these compounds are able to permeate the stratum corneum, and so to penetrate into deeper skin layers[2,3]. Preparations of *C. officinalis* are mainly applied in the form of infusions, tinctures and ointments as wound healing remedies for inflammation of the skin, mucous membranes, to prevent infection and to soothe inflamed and damaged skin[4], but

also for poorly healing wounds, bruises, boils and rashes, e.g., pharyngitis and leg ulcers[5]. In the mixed lymphocyte reaction, 70% ethanol extract showed stimulatory effects at 0.1-10 µg/ml, followed by inhibition at higher concentrations[6]. Human granulocytes phagocytosis was stimulated by polysaccharides isolated from *Calendula* flowers aqueous extract[7]. Extracts of *Calendula* flowers of differing polarities exhibited anti-oxidative effects on liposomal lipid peroxidation induced by Fe^{2+} and ascorbic acid[8,9]. Isorhamnetin 3-glycosides from *Calendula* flowers inhibited lipoxygenase from rat lung cytosol at a concentration of 1.5×10^{-5} M[10]. In a test system based on porcine buccal membranes, strong concentration-dependent adhesive processes were observed with a low viscosity polysaccharide enriched extract (98% carbohydrates) of *Calendula* flowers. These findings suggest that the polysaccharides may contribute to therapeutic effects in the irritated mucosa treatment[11]. Lycopene, saponins, triterpenoids and flavonoids, like quercitin prevent from releasing of harmful and histamine enzymes, which cause sensitivity and inflammation, heal the redness and pain, and inhibits plasma release to the tissue, decreasing the capillary permeability. At the same time, terpenoids reduce the immigration of white blood cells into inflamed area[12] (*Eghdampour et al., 2013*) and lycopene consistently reduces transcript levels of pro-inflammatory cytokines[13]. In animal studies, *Calendula* has showed to stimulate granulation and increase glycoproteins and collagen at wound sites[14,1]. *Calendula officinalis* extract could enhance the collagen content whether by increasing synthesis or decreasing catabolism of collagen[1]. A fraction enriched in triterpene administered orally to mice inoculated with Ehrlich mouse carcinoma prevented the as cites development and increased survival time compared to control[15].Triterpeneslikefaradiol and taraxasterol inhibit experimental tumor promotion and are consequently considered inhibitors of tumor growth[16]. A saponin rich fraction given orally at 50 mg/kg body weight to hyperlipemic rats reduced the serum lipid level[17,18].Oral administration of *C. officinalis* extract is effective to inhibit serum glucose levels increase in glucose-loaded mice; gastric emptying and finally shows a potent gastro-protective effect in rats, inhibiting ethanol and indomethacin-induced gastric lesions in this one[19]. The aqueous alcohol extract of *C. officinalis* exhibited central nervous system inhibitory effect with marked overall sedative activity as well as hypotensive effect[20]. The alcohl extract of *C. officinalis* flowers, instead, possesses anti-HIV properties, it seems that may inhibit early events in the HIV replication cycle, suppressing cell fusion[21]and is also able to inhibit the Epstein-Barr virus early antigen activation[22] and to suppress the replication of herpes simplex *in vitro*[23]. A cream containing *Calendula* extract has been showed to be effective in dextran and burn edemas as well as in acute lymphedema in rats. Activity against lymphedema was primarily ascribed to macrophage proteolytic activity enhancement[24]. The flowers essential oil inhibited the growth *in vitro* of *Bacillus subtilis*, *Escherichia coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Candida albicans*[25,26]. Acetone, ethanol or water extracts inhibited the growth *in vitro* of the fungus Neurospora crass[27]. A flavonoid fraction isolated from the flowers inhibited the *in vitro* growth of *S. aureus*, *Sarcinalutea*, *E. coli*, *Klebsiella pneumonia* and *Candida monosa*[28].The 50% ethanol extract of the plant showed spermicidal activity in rats at 2% concentration[29]. Flavonoids and saponins prevent from releasing of harmful and histamine enzymes,

which cause sensitivity and inflammation; heal the redness and pain; and inhibit plasma discretion to the tissues by decreasing the capillary permeability. Triterpenoids provide the anti-inflammatory activity. The essential oil from *C. officinalis* flowers exhibits good sun protection activity due to its antioxidant activity[30]. Cutaneous application of a cream containing essential oil of *Calendula* (at 4% and 5%) prevents UV-B-induced alterations in the level of antioxidants in rat skin tissue, with increasing in superoxide dismutase (SOD), catalases (CAT), ascorbic acid level (ASC) and total protein (TP) level, and simultaneously reduction in malondialdehyde (MDA) content, a marker of lipid peroxidation[31]. The photo-protective effect of topical gel formulation of *C. officinalis* is thought to be associated with an improvement in collagen synthesis in the sub-epidermal connective tissue, topical application accelerates the biological events involved in the photo-damaged dermal healing process[1,3]. Furthermore the presence in the *Calendula* extract of β-carotene, quercitin[13], 1,8-cineole and α-pinene, compounds with scavenge free radicals activity, conferring it a good antioxidant potential[2,31]. In a randomized, open controlled study, were compared the effects of three ointments after topical treatment of patients with 2nd or 3rd degree burns for 17 days: *Calendula* flower ointment (prepared by digestion in vaseline) (n = 53) or vaseline only (n = 50) or a proteolytic ointment (n = 53). The success rates were considered to be 37/53 for *Calendula* flower ointment, 27/50 for vaseline and 35/53 for the proteolytic ointment[32]. In an open uncontrolled pilot study, 30 patients with burns or scalds were treated 3 times/day for up to 14 days with a hydrogel containing 10% of a hydro-ethanol extract. The symptoms like reddening, swelling, blistering, pain, soreness and heat sensitivity were scored before, during and at the end of treatment. Total score and individual scores for each symptom improved[33]. Local application of a mixture containing 70% oily extract of *Hypericumperforatum* and 30% oily extract of *C. arvensis*in women with surgical wounds improved the rate of healing, compared with controls[34].In a phase III randomized single blinded trial *C. officinalis*is compared with trolamine in the prevention assessment of acute dermatitis during irradiation for breast cancer. Patients who had been operated on for breast cancer and who were to receive post-operative radiation therapy were randomly allocated to application of either *Calendula* ointment containing 20% of fresh *Calendula* aerial parts in petroleum jelly (126 patients) or trolamine (128 patients) on the irradiated fields after each session. The primary end point was the occurrence of acute dermatitis of grade 2 or higher. Secondary end points were the occurrence of pain, the quantity of the topical agent used and the patient satisfaction. The occurrence of acute dermatitis of grade 2 or higher was significantly lower (41% vs. 63%; $p< 0.001$) with the use of *Calendula* than with trolamine. Moreover, patients receiving *Calendula* had less frequent radiotherapy interruption and significantly reduced radiation-induced pain[35].Furthermore the clinical relevance of this finding is emphasized by the significant improvement in self-assessed patient satisfaction with regard to pain and dermatitis[35]. Another study led to healthy volunteers has reported that *C. officinalis* as a cream preparation protects skin from irritant contact dermatitis caused by exposure to sodium lauryl sulfate[36]. In 34 patients with venous leg ulcer was carried out clinical examination of an ointment with *C. officinalis* extract. A total of 21 patients with 33 venous ulcers were treated with ointment, applied twice a day for 3 weeks. Control group that consisted of 13 patients with 22 venous

ulcers were treated with saline solution dressings, applied to ulcers for 3 weeks. In the experimental group, the total surface of all the ulcers at the beginning of the therapy was 67,544 mm^2. After the 3rd week, the total surface of all the ulcers was 39,373 mm^2 (a decrease of 41.71%). In seven patients, complete epithelialization was achieved. In the control group, the total surface of all ulcers at the beginning of the therapy was 69,722 mm^2. After the 3rd week, the total surface of all ulcers was 58,743 mm^2 (a decrease of 14.52%). In four patients, complete epithelialization was achieved. There was a statistically significant acceleration of wound healing in the experimental group ($p< 0.05$), suggesting the positive effects of the ointment with marigold extract on venous ulcer epithelialization[37].Schmidgall and coworkers (2000)[11] evaluated on an experimental *ex vivo* pig mucosal patch the bio-adhesiveness of herbal polysaccharides extracts to the epidermal layer, confirming that *Calendula officinalis* has this specific property[11].

In a recent study of Tedeschi *et al*. (2012)[38], *Calendula officinalis* extract was enclosed in a gel formula including isoflavones, *Lactobacillus sporogenes*, and lactic acid and challenged to 186 women mean age 53.7 years, postmenopausal for 4.1 years, using standardized oral isoflavones, 103 of which introducing the vaginal gel and 83 without topical treatment. The itching, burning, vulvovaginal erythema, vaginal dryness and dyspareunia intensity were compared in the two groups; the use of the gel gave a definite symptoms relieve compared with no local treatment[38]. Eghdampour *et al*. (2013)[12] evaluated the effect of *Aloe vera* and *Calendula* on perineal episiotomy healing with a randomized clinical trial involving 111 primiparous women. They were randomly categorized into three groups of control (n=1) and experimental (n=2) groups. The women in experimental group used *Aloe vera* and *Calendula* ointment every 8 hours and the control group used hospital routine on episiotomy for 5 days[12]. The data were collected by demographic questionnaire and redness, edema, ecchymosis, discharge and approximation scale (REEDA) which investigated the episiotomy healing before and five days after intervention in two groups. REEDA scale in five days after delivery showed statistically significant difference between control and experimental groups. *Aloe vera* and *Calendula* ointment considerably increase independently, the speed of episiotomy wound healing[39]. Another protocol has been published randomly comparing topical *Aloe Vera* against *Calendula Officinalis*on diaper dermatitis in 63 children and infants with a protocol encompassing 3 cream delivery /daily for 10 days. The severity of dermatitis was graded at baseline as well as at the end of trial using a 5-point scale. The adverse effects of study medications were assessed during the trial. Both treatment groups ($p<0.001$) had positive results on the symptoms but *Calendula* ointment improves significantly the number of rash sites compared to *Aloe* group ($p =0.001$). No adverse effect was reported from either of the medications.

Lavagna and colleagues (2001)[34] estimated the benefits of *Hypericum* and *Calendula* oils in the epithelial reconstruction of surgical wounds in 24 childbirth with caesarean section, stating that the combined compounds were very effective in reducing the wounds diameter[34].

Calendula officinalis hydroalcoholic extract has not shown acute toxicity following administration in rats and mice[21,23]. The main adverse event is allergic contact dermatitis[40,23]. No serious adverse effects have been signaled, and it is considered safe to use both topically and

orally[14,31], even though systemic administration of marigold products should be avoided during early stages of pregnancy because of its ability to stimulate menstrual flow[41].

Melaleuca alternifolia (Tea Tree oil)

Melaleuca alternifolia essential oil (*Myrtaceae*) is used since ancient time for treating a wide variety of medical diseases and conditions. It -is promising as an antimicrobial and is effectiveness in a several of dermatologic condition including dandruff, acne, head lice, herpes and other cutaneous infections like tonsillitis, stomatitis and vaginitis[42,43]. Tea tree oil is currently incorporated into various range of pharmaceutical and cosmetic products for the treatment of sores, blisters, spots, rashes, warts, burns and acne[44]. Tea treeoil (TTO) contains approximately 100 compounds, mainly terpene hydrocarbons, monoterpenes, sesquiterpenes and their corresponding alcohols[14,45] as well as aromatic compounds. Many of this compounds penetrate skin and enhance the penetration of other substances, this may boost TTO medical effects, but it may also potentiate the opportunities of toxicity[46].The composition of tea tree oil has been regulated by the International Organization for Standardization (Oil of Melaleuca-terpinene-4-ol type) (ISO4730:2004), that specifies compositional limits for 15 of the approximately 100 compounds[47,45,48]. Tea tree oil is comprised of at least 30% terpinen-4-ol which causes most of its biological activity, although in practice this is often approximately 40% of the oil and a maximum content of 15% of 1,8-cineole[49].

Terpinen-4-ol and α–terpineol, the mainly bioactive TTO compounds, show anti-inflammatory activity when applied topically in a carageenan-induced hind paw oedema rat model[50]. Furthermore terpinene-4-ol has been demonstrated that TTO may selectively regulate cell function during inflammation, in particular monocyte activity and after topical application it may regulate inflammatory responses to foreign antigens in the skin; then it is able to repressmonocyte superoxide production and consequently it prevents oxidative tissue damage that may be seen in more chronic inflammatory states[51]. A clinical study has reported that TTO lid scrubbing is able to essentially reduce the tear levels of inflammatory cytokines (IL-1β, IL-17) involved in various immune response and inflammatory processes in ocular demodecosis patients[52]. Several *in vivo* studies have shown that TTO has inhibitory effects on inflammatory processes, such as reduction of histamine-induced oedema and contact hypersensitivity after topical TTO application in mice[53,54]. Furthermore, clinical studies of human volunteers have demonstrated that TTO can reduce significantly the histamine-induced skin inflammation (developing oedema histamine-induced)[55] and nickel-induced contact hypersensitivity in human dorsal skin after topical application of 100% TTO[56]. Moreover, TTO hydrogel exhibits effectiveness in cooling burn wounds and in increasing the rate of wound healing in a porcine model[57]. In a clinical study led on 49 humans with chronic gingivitis, Soukoulis and Hirsch (2004)[58] have reported the efficacy of topically application of TTO-containing gel in reducing gingival inflammation[58].

Tea tree oil has also antiviral activity: *in vitro* studies have demonstrated the TTO virucidal activity and TTO efficacy in viral plaque formation reduction against *herpes simplex* virus (HSV-1 and HSV-2) and *influenza* virus subtype H1N1. It seems that TTO interferes with an early step of the viral replicative cycle[59]. Furthermore the TTO antiviral activity has been confirmed in a randomized, placebo-controlled trial on

patients with self-reported history of recurrent *herpes labialis*[60]. At a later stage Millar and Moore (2008)[61] have reported the successful of TTO treatment in pediatric patients with warts on their right middle finger. In this trial TTO was applied topically once daily to the lesions for 12 days with a favourable outcome, including complete re-epithelization of the infected areas, highlighting the potential use of TTO in the treatment of common warts due to *human papilloma* virus (HPV)[61].

Greay *et al*. (2010)[62] have described for the first time TTO antitumor activity. They have shown that topical 10% TTO/DMSO, thanks to its abundance in terpenes, has *in vivo* antitumor activity, inducing regression and growth inhibition of aggressive, subcutaneous, chemo-resistant, melanoma mice model, highlighting topical TTO as a possible alternative topical antitumor treatment[62]. Afterwards, Bozzuto *et al*. (2011)[63] have supported this finding, showing that TTO is able to inhibit the growth of melanoma cells and it is even able to overcome multidrug resistance (MDR), indicating that TTO can also interfere with the migration and invasion processes of drug-sensitive and drug-resistant melanoma cells[63].

Tea tree oil is widely used topically for the treatment of bacterial and fungal infections because of its broad antimicrobial activity against several strains of bacteria, fungi and protozoan. The antimicrobial activity of TTO has been proved against many organisms, including *Staphylococcus aureus, coaugulase-negative staphylococci, Staphylococcus spp.* (methiccillin-resistant and –-sensitive *S. aureus* MRSA and MSSA), vancomycin-resistant *enterococci, Acinetobacterbaumannii, Escherichia coli, Propionibacterium acnes, Staphylococcus epidermidis, Klebsiella pneumonia, Candida albicans, Candida spp., Trichophytonspp, Malessezia furfur and Trichomona-svaginalis*. The wide range of TTO susceptible organisms proposes that this agent may be useful for skin antisepsis and to facilitate healing in chronic *Staphylococcus*-infected wounds. Moreover many pathogenic organisms have been shown to be more susceptible to tea tree oil than commensal organisms[64,46,65]. Clinical studies have demonstrated the efficacy of percutaneous application of TTO in resolution of osteomyelitis due to MRSA infection in adult male[66], and MRSA-infected wounds, showing fast healing, reduction in inflammation, pain and wound odor. Furthermore TTO appeared more efficacious than the standard treatment and better tolerability by patients[65]. In a recent study Malic *et al*. (2014)[67] have tested and proved the TTO biocide activity against urinary catheter pathogens (several strain of *Proteus mirabilis* and *Proteus vulgaris*) suggesting that TTO may be a good alternative approach to incorporating into biomaterials used in catheter development to inhibit growth and swarming of *P. mirabilis* in long-term-catheterized patients[67]. TTO antibacterial activity has been proved also against *mutans streptococci* and other oral microorganism in a clinical study to compare the antimicrobial activity of tea tree oil and chlorhexidine solutions against oral microorganisms[68,69]. Beforehand, Jandourek *et al*. (1998)[70] have been demonstrated the efficacy of TTO oral solution as an alternative regimen for AIDS patients with oropharyngeal candidiasis refractory to fluconazole[70]. The fungicidal activity of TTO has been confirmed also by Ramage *et al*. (2012)[71] in a study in which they evaluate the efficacy of TTOagainst *C. albicans* clinical strains isolated from a variety of patients, to support the forcefulness of a putative application of TTO in prophylaxis and treatment of oropharyngeal candidiasis[71]. A multicenter, randomized controlled trial have reported

that tea tree oil is highly effective in the treatment of superficial fungal infections such as onychomycosis[72]. In this last study on 117 patients, it has been compared a 100% tea tree oil solution with 1% clotrimazole solution in the treatment of onychomycosis. After 6 months of treatment, the 2 groups showed comparable results on the basis of mycologic cure (11% for clotrimazole and 18% for tea tree oil) and clinical assessment and subjective rating of appearance and symptoms (61% for clotrimazole and 60% for tea tree oil)[72]. A previous clinical study has reported that a 10% tea tree oil cream works about as well as tolnaftate 1% cream in treating symptoms of athlete's foot, although being less effective than clotrimazole or terbinafine[73]. Later in a randomized, controlled, double-blinded trialto determine the efficacy and safety of 25% and 50% tea tree oil in the treatment of interdigital tinea pedis, Satchell *et al*. (2002)[74] have reported that there is a marked clinical response seen in 68% of the 50% tea tree oil group and 72% of the 25% tea tree oil group, compared to 39% in the placebo group after 4 weeks of treatment[74]. These data suggest that TTO may have a role in at least symptomatic treatment of tinea pedis and onychomycosis and other superficial wounds. Furthermore, *in vitro* studies have demonstrated the TTO efficacy against various dermatophyte species including *Malessezia species*, the etiological agents in many superficial skin diseases such as seborrheic dermatitis, suggesting that tea tree oil ointment may be a good alternative in the therapy of fungal infections of the skin and mucous membranes as well as in the treatment of dandruff, a mild form of seborrheic dermatitis[75,76].

A recent phytotherapy review onthe issue: “vaginitis” addressed to find new trends in pharmacological treatments, outlined the role of tea tree oil including terpinen-4-ol, alpha-terpinene, gamma-terpinene and alpha-terpineolas effective anti-bacterial, anti-fungal and anti-protozoal compounds; previous *in vitro* studies[77]. Preceding study has reported that treatment with TTO vaginal pessaries of bacterial vaginosis patients was effectiveness in removing symptoms without affecting vaginal flora commensal *lactobacilli*, suggesting a target effect of tea tree oil against pathogenic microorganisms. These data have been confirmed by Hammer *et al*. (1999)[78] in a study in which they tested tea tree oil susceptibilities of lactobacilli and organisms associated with bacterial vaginosis. They reported indeed that all *lactobacilli* tested are appreciably more resistant to tea tree oil then organisms associated with bacterial vaginosis[78]. Mondello *et al*. (2006)[79]on experimentally infected oophorectomized rats confirmed the positive fungicide effect of terpinen-4-ol, the main bioactive component of *Melaleuca alternifolia* Cheel against azole sensitive candida species. *In vitro* and *in vivo* studies have showed the efficacy of TTO in the treatment of vaginal candidiasis in rat experimental model. These studies have demonstrated that TTO has fungicidal dose-dependent activity against fluconazole/itraconazole-susceptible and -resistant strains of *Candida albicans* and against *C. glabrata, C. neoformans and C. parapsilosis*. The researchers have also reported that TTO effectiveness is comparable to that of fluconazole in drug-susceptible organisms and is not influenced by low pH. These findings suggest that TTO may be a putative therapeutic agent in the treatment of acute and recurrent forms of vulvovaginitis[42,79].

Banes-Marshall *et al*. (2001)[80] explored the antibacterial antifungal action of tea tree oil against methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus*, *faecal streptococci*, *beta-haemolytic*

streptococci, *coagulase-negative staphylococci*, *Pseudomonas spp.* and *coliform bacilli*. Eleven *Candida spp.* isolated from skin and vaginal swabs. In 64 specimens TTO produced an inhibitory and bactericidal effect at 3% and 4% (v/v), respectively. *S. aureus* and *Candida spp*. were the most susceptible to TTO, with MICs and MBCs of 0.5% and 1%, respectively. *P. aeruginosa* and the *faecal streptococci* isolated, with MICs and MBCs of >8%, were resistant to TTO[80].

Watson and coworkers (2012)[81]performed a sixty-six health professionals (medical practitioners, dermatologists, nurses and allied health professionals) survey to evaluate the quality of complementary and alternative prescriptions in recurrent vulvovaginal candidiasis; the caregivers in the medical and ob/gyn area collected info about complementary alternative medicine (CAM) management enclosing lactobacillus, which was the most self-described, oral and vaginal yogurth, vinegar, garlic, Chinese medicine and tea tree oil; TTO was documented to be largely used in the folk medicine, even if *Lactobacillus* was the most common remedy reported in this survey[81].

Traditional evidence from almost 80 years of use indicates that topical use of tea tree oil is relatively safe, and that adverse events are minor and occasional in absence of ingestion[82]. There are few reports of allergy and contact dermatitis in predisposed individuals[47,49,82]; evidences of poisoning if taken internally in higher doses and skin irritation at higher concentrations[46,47]. However, degradation products of monoterpenes in tea tree oil actually appear to be sensitizing agents. It seems that the prevalence of allergy is approximately 5% [76]. Therefore TTO topical treatment is considered safe[14;82], though the European Cosmetic Toiletry and Perfumery Association (COLIPA) in 2002 has recommended that tea tree oil should not be used in cosmetic products in a way that results in a concentration greater than 1% oil being applied to the body[44] (*SCCP, 2004*). Adverse reactions may be minimized by avoiding ingestion, applying only diluted oil topically, using oil that has been stored correctly or using only the TTO isolated bioactive ingredients[47].

Salvia officinalis Linn (Sage)

Salvia officinalis L.(Lamiaceae) is a Mediterranean species, naturalized in many countries.The traditional medical experience comprises several thousand years of internal and external application and nowadays the preparation of dried leaves of *S. officinalis* is registered as a medicinal drug[83,84]. *Salvia officinalis* has been used for encouraging healing, excessive menstrual bleeding, hot flashes (menopausal sweats) hyperhidrosis, vaginal discharge and improving memory[85]. In Jordan, it is used in traditional medicine as antiseptic, antiscabies, antisyphilitic, and anti-inflammatory, being frequently used against skin disorders. It is applied both externally and internally as anti-phlogistic and astringent drug[84]. The main compounds of *S. officinalis* oils were 1,8-cineole (39.5-50.3%) and camphor (8.8-25.0%) alpha-thujone, beta-thujone, borneol, and viridiflorol and phenolic diterpenesCarnosic acid (CA) and carnosol (CS) which are inhibitors of Peroxisome proliferator-activated receptor gamma (PPARgamma), a ligand activated transcription factor, belonging to the metazoan family of nuclear hormone receptors. Activation of PPARgamma increases the transcription of enzymes involved in primary metabolism, leading to lower blood levels of fatty acids and glucose. Animal experiments and *in vitro* studies have reported that *Salvia officinalis* extracts may significantly decrease serum glucose in diabetic rats and

normoglycemic mice[86,87];furthermore it is safe and effective in the improvement of lipid profile, including a decrease on the highly atherogenic LDL-C particles and an increase in the HDL-C particles in healthy humans; of antioxidant defenses, increasing superoxide dismutase (SOD) and catalase (CAT) activities in human erythrocytes; and of lymphocyte Hsp70 protein expression in human volunteers, contributing to stress tolerance and cytoprotection[87,88].

The anti-inflammatory and antiphlogistic properties of *Salvia* extract are due to triterpenesoleanolic, ursolic acid and diterpenecarnosol. A study that investigates topical anti-inflammatory activity of *Salvia officinalis* leaves, obtained from four plant populations of different origins extracted in two different ways, revealed that ursolic acid is sage component that exhibits the stronger anti-inflammatory properties and it could explain anti-phlogistic activity of the official plant drug *Salviae folium*. In this last report anti-inflammatory activity of the two sage extracts is tested by evaluation of extracts inhibiting ability of the Croton oil-induced ear oedema in mice. The anti-inflammatory effect of ursolic acid is compared with that of a reference non-steroidal anti-inflammatory drug, indomethacin. The ursolic acid activity appears two fold more potent than that of indomethacin[89].

The antibacterial properties of sage oils have been attributed to the presence of 1,8-cineole, thujone, and camphor[90]. However in 2007, Pinto *et al*.[91] declaredthat thujones do not play an important role against yeasts and filamentous fungi. This work also suggests 1,8-cineole and camphor as the main responsible for the antifungal activity in the tested strains91. A randomized, double-blind, placebo-controlled study has demonstrated clinical efficacy of a spray, containing 15% of a *Salvia* fluid extract, in the symptomatic pain management of acute viral pharyngitis[88,92]. The astringent activity of sage (active ingredient of dental-care herbal medicinal preparations) promotes the reduction in plaque growth, the inhibition of gingival inflammation and has positive effects on caries prophylaxis[93]. Furthermore, gurgle solutions with sage leaf are typically applied in acute laryngopharyngitis[94]. Many studies have shown that *Salvia officinalis* essential oil exhibits strong or moderate antimicrobial activity against various strains of bacteriaand yeast, including *Candida albicans* and dermatomycetes, depending on sage chemical composition[95,96,97]. Mosafa *et al*. (2014)[98] have led a study to test antibacterial activity of sage ethanol extract through well diffusion and microdilution method against multidrug resistant bacteria. In this study they have showed that not only essential oil has higher antimicrobial effects, but also ethanol extract of sage leaves and stems have antibacterial effects both Gram negative and Gram positive multidrug resistant bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, *Pneumoniae aeruginosa* and *Klebsiella pneumoniae*. They have also reported that sage antimicrobial activity is dose-dependent, with increased effect increasing concentration[98]. *Salvia officinalis* oil is equally useful at countering bacterial infections, since it kills bacteria and inhibits their growth. This property can also be used to heal ailments like bacterial infections in the ears, nose, throat, eyes, genitals, urethra, colon, intestines as well as on the skin and in wounds. This oil is also capable of inhibiting fungal infections both internally and externally, and gives relief from fungal infections like dysentery, skin diseases, Athlete's Foot, or dermatitis. In a study of Abu-Darwish (2013)[99], the oils revealed antifungal activity against dermatophyte strains and significantly inhibited inflammation generated by NO production

LPS-induced in macrophages, without affecting cell viability, in concentrations up to 0.64 µL/mL. This is the first report regardingthe *in vitro* anti-inflammatory potential of *S. officinalis* oil. These findings demonstrated that mammalian macrophages and keratinocytes viability is not affected by bioactive concentrations of *S. officinalis* oilmaking itsuitable to be incorporated in skin care formulations for cosmetic and pharmaceutical purposes[99]. In the clinical setting a wide observational study of Guaschino and Benvenuti (2008)[100] reviewedvaginalpH, lifestyle and correct intimate hygiene in women of different ages and in different physiopathological conditions. Different randomized subgroups of 2641 women (prepubertal, fertile, pregnancy, breastfeeding, premenopause and menopause). In this survey, the most appropriate detergent for the woman's intimate hygiene was recommended by 264 gynecologists on the basis of age and physio-pathological status of the patients, and extract of *Salvia officinalis*to be used once or twice a day for four weeks was mainly, spontaneously prescribed for adolescents, child-bearing age and pre-menopause; this investigation highlights the traditional role of *Salvia* in routine female intimate hygiene like the natural plant extracts favoring the reduction of vaginal pH, the improvement of symptoms and the quality of sexual activity in all age/conditions observed[100].The estrogenic activity of *Salvia officinalis* has been demonstrated experimentally and a team of investigators has shown the herb has strong effects in cases of oligomenorrhea and amenorrhea; therefore, sage can be used for hormone imbalances as premenstrual tension, endometriosis, cysts and for stimulate, regulate and promote a normal menstrual flow. It seems that sage tea drinking, via hypothalamic-pituitary-ovarian axis, may be improves female fertility in adult rats[101]. Sage efficacy for the treatment of hot flushes during menopause and of the associated menopausal symptoms (e.g. insomnia, irritability, dryness of vagina etc.) was demonstrated in an open multicenter clinical trial[88,102]. Conserning the anti-fushes *Salvia* extract effect, Rathe *et al*.(2013)[103] identified with the vacuum liquid chromatography, luteolin-7-O-glucuronide (EC_{50}129 µg/mL) as the active putative estrogenic principle; flavonoids are safe and commonly used herbal medicinal product during the menopause involved in the anti-hot flush effect of *Salvia officinalis*[103].

Several studies have shown that the consumption of *Salvia officinalis* extract is safe and non-toxic[88,92,102,104,105].

CONCLUSION

Rationale for the combined formula of the hydroxypropilmetilcellulose and 3 herbal extracts in a vaginal gel.

The combination of HPMC and *Calendula*, *Melaleuca,* and *Salvia Officinalis* in a single topical delivery system is a unique opportunity to face vaginal care with an exhaustive integrated mucosal defense strategy. Basically the vaginal epithelium is exposed to different and complexes stressor agents producing epithelial disruption, inflammation and cancer, especially when the hormonal trophic action is reduced by the advancing menopause; recurrent parasitic infections, such as *Trichomonas,* bacteria, fungi and viruses contagions, when vaginal pH has been modified to basic levels alter the microflora, inducing dramatic inflammatory reactions, often turning to chronic stage, that may lead to epithelial dysplasia.

The action mechanism of the 3 herbs extracts in combination, takes control of the vaginal surface integrity and mucosa restoration after mechanical or chemical or microbiological damage, especially through the well-known *Calendula* contributes to

body defenses against external agents, has anti-inflammatory, antioxidant and healing properties; *Salvia* expresses altogether a wide and selective control, supports the natural mechanism for body´s purification, promotes the toxin elimination; *Melaleuca* expresses altogether a wide and selective control of pathogenic microflora enclosing the often relapsing *Candida albicans* super-infections. Furthermore, *Salvia* and *Calendula* promote an estrogen –like trophic effect upon the vaginal mucosa, enhancing the natural lubricating properties of the mucins secretion. Obviously the modern drug-manufacturing technology strongly emphasizes the quality of such a gel with long standing bioavailability upon the vaginal mucosa, and of the mixed herbal extracts with specific and well balanced eutrophic and infections preventing properties; HPMC has been proven a quite effective background for active principles delivery, and lactic acid stabilizes the acidic pH of the medium promoting homeostasis of indigenous microflora.

The table 1 summarizes the contribution of each active principle to maintenance of vaginal mucosahomeost as is through protective, lubricant and soothing functions.

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Table 1. Female genital wellness and disease prevention

PROTECTIVE			LUBRICANT		SOOTHING		
Maintenance of vaginal microflora			Estrogenic-like property		Anti-inflammatory		
Melaleuca	*Calendula*		*Calendula*	*Salvia*	*Melaleuca*	*Calendula*	*Salvia*
Antimicrobial property			Trophic effect		Antioxidant		
Melaleuca	*Calendula*	*Salvia*	*Calendula*	*Salvia*	*Calendula*	*Salvia*	*Melaleuca*
Antioxidant			Moisturizing		Healing property		
Calendula	*Salvia*	*Melaleuca*	*Calendula*		*Calendula*	*Melaleuca*	