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Development and Evolution of Herbal Intimate Hygiene Germicide Product for Women's Hygiene without any Side and Adverse Effect in an *In vitro* Study

Mukta Jain [a*]

[a] *S. S. Jain Subodh P. G. Autonomous College, Rambag, Jaipur, Rajasthan, India.*

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Vagina is the primary mode of sexual transmission of microbes and infection. Women frequently use feminine hygiene products as part of their daily cleaning habits but most of them are not aware about harmful, adverse and side effects of these synthetic hygiene goods. This article provides context on the herbal germicide-aided natural solution for women's hygiene. For that purpose we use Withania Somnifera plant with other easily available plants, identify their plant extract's chemicals by gas chromatography, find their bactericidal, fungicidal and germicidal properties by standard methods. To ensure completion of our products we use molecular weight determination IR, NMR, MASS and 13 carbon spectroscopy .we followed stepwise process of developing herbal hygiene product i.e. plant materials collection, extraction of crude by hydro distillation method, characterization of crude extract by gas chromatography and gas chromatography mass spectroscopy techniques separation the bioactive compounds by HP_TLC and HPLC, purification by HPLC, the designed product have characterized by GC and GCMS

Keywords: Female hygiene; germicide; intimate; natural; herbal; microbiota; In vitro study.

**Corresponding author: E-mail: drmuktajain1@gmail.com;*

1. INTRODUCTION

Feminine hygiene is still understood as a taboo or transgressive subject, but it is an important part of women's self-care and health. Feminine hygiene is common adoption for women of all ages to perform sanitary habits throughout and after menopause. Women's health items such as washes, wipes, and sanitary napkins are often used by females to maintain their personal cleanliness. Numerous synthetic chemicals or cleansers are involved with various washes or sanitary items under these procedures.

All these synthetic germicides or toxins kill 100% of germs, bacteria, and viruses and also artificially make the genital pH drop below a critical level, which is not good from a health aspect. These actions by the commonly used hygiene products make the genital area more vulnerable to infections, including sexually transmitted diseases. Similarly, females utilize feminine hygiene goods such as sanitary napkins, tampons, and cups that contain BPA or other pesticide chemicals, during their menstrual cycle. With a time restriction, such feminine hygiene products soak or collect bloody discharge and other fluids but have adverse effects on the female reproductive system. Even a mild infection can lead to cervical cancer, which can be fatal if it enters the bloodstream and damages the reproductive organs of women as a whole. Currently, there are certain herbal remedies which are used only during menstruation, although they may also induce unpleasant product experiences, allergic reactions, extreme dizziness etc. In the process even if some modification is gained, cost becomes higher. Such high costs render these methods of limited applicability, if at all. Thus, effective alternative and herbal germicide-aided natural solutions are needed for feminine hygiene. Therefore, the main objective of the project is to formulate herbal germicide-aided natural solution usable during regular daily life as well as the menstruation cycle.

2. REVIEW OF LITERATURE

Normally, the female vagina produces a mucus secretion from the cervix and vagina. This mucus functions as a natural cleaner to maintain moisture and avoid dryness and irritation. Through the generation of lactic acid by Lactobacillus species (*L. crispatus, L. iners, L. jensenii,* and *L. gasseri*) in the vagina, this typical discharge simultaneously creates pH values in the range of about 3.8 to 4.5, providing an acidic environment, [1,2]. This normal discharge is clear and doesn't smell at all. The consistency and amount of a woman's vaginal discharge change at different times in her life and depend on the time of her menstrual cycle. But things like hormone medications, having more than one partner, using oral contraceptives, taking antibiotics for infections in other parts of the body, using condoms, smoking, and bad hygiene habits change the vaginal pH balance and make it easier for vaginal pathogens to grow [3,4]. At the age range of 46-52 and high levels of follicular stimulating hormone (FSH) also *cause* a rise in pH in the menopausal/postmenopausal stage [5-7]. It is distinguished by a transition away from microflora that is dominated by Lactobacillus toward those that include a more diversified variety of facultative and obligate anaerobic microbes [8]. *Atopobiumvaginae, Gardnerella vaginalis, Megasphaera spp, Staphylococcus aureus, Streptococcus bovis, Staphylococcus epidermidis, Helicobacter pylori, Mycoplasma hominis, Ureaplasmaurealyticum*, *Trichomonas vaginalis*, *Candida albicans*, etc. are some of the microbes that cause infections with bad-smelling discharge at Female genital system [9-16]. According to a study, about 50% of women have Candida in their vaginas because of their immune systems, even though they don't have any symptoms (Qi et al. 2021). Studies show that 20% of asymptomatic, healthy childbearing women have Candida in their vaginal tract [17]. In consequence, menstrual blood in the vagina causes a rise in protein concentration as well as oxygen and carbon dioxide concentrations, and a reduction in vaginal lactobacilli, all of which contribute to the colonization of uropathogenic microbes [18]. In such cases of gynaecologic infections, several treatment methods based on antibiotics or chemicals are used both for therapy and prophylaxis. Traditionally, these medicinal products used for vaginal application are mostly applied superficially for the skin penetration of certain gynaecological pathology, such as candidiasis, genital herpes, and vaginitis [19]. Most often, antibiotics metronidazole and clindamycin, given orally or vaginally, or both, are suggested solely for women who are exhibiting symptoms [20,21]. However, it was reported in a study that after successful antibiotic delivery, reinfection developed due to biofilm formation on the vaginal dermis [22 ,23].

Modern women have revolutionized her lifestyle, moral etiquette, and personal hygiene. It has

been reported that a majority of women prefer to use toilet paper, soaps with varying pH levels and intimate deodorants, as well as sanitary pads, panty liners and the frequency of sexual intercourse. In their everyday lives, they choose soap with shower or intimate wash to clean their intimate regions and avoid infection [24-26].

The problem is that there is less evidence available on the full scope of the health concerns linked with the large variety of vaginal washing solutions. This is the area that frames the scope of this project. However, studies have shown that women who use lubricants, deodorant sprays, synthetic wash, and powders for vaginal washing may be more susceptible to urinary tract infections, bacterial vaginosis, sexually transmitted infections, and an increased risk of ovarian cancer [27-33,]. For instance, *H. pylori* infection is a good example of a disease that may be prevented with proper hygiene practices or sexual activity, and if transmission has occurred during infancy, it can be stopped by adopting new behaviours [34,35]. Similarly, another research [36] demonstrated that pregnant women with practise of not washing hands after going to the toilet or genitals after coitus exhibit *Urinary Tract Infection*. The decrease of vaginal pH below the extensive threshold is associated with disturbances in the vaginal microbiome as a mechanism through which certain vaginal washing products create health issues. This disturbance may result in reproductive tract infections or infertility in women [37-42]. According to research, intra-vaginal cleaning agents alter the microbiome by eliminating beneficial bacteria and mucus from the vaginal canal [43]. If the infection extends, it may lead to life-threatening conditions such as ascending infections, cervical Ulcers, cervical cancer, spontaneous abortion, low birth weight babies, and even miscarriage [44].

According to Society of Gynecologists and Obstetricians of Canada, and the United States Office of Women's Health and opinions of Vaginal hygiene specialists, the suggestion is that the use of deodorants with fragrance and douches/Intimate wash might irritate the vaginal area [45,46]. Ultimately, any product that has the power to modify the mucosa or pH of the vagina may do more damage than benefit and should be avoided.

On the other hand, certain herbal products are available and used for female hygiene. A case study by Ozen and Baser [46,47] demonstrated that apple vinegar was a very effective antibiotic and disinfectant against vaginal candidiasis infection. In a research conducted by Nakagawa et al. [48], rosemary extracts were shown to be the most efficient against S. aureus recovered from clinical isolates. In addition, research [49] indicated that phytotherapeutic compounds of *Pistacialentiscus mastic* and *Ocimumbasilicum* efficiently demonstrate anti-*Trichomonas vaginalis* efficacy. The *polyherbal microbicide 'BASANT', formulated at Obstetrics & Gynecology clinic at Jawaharlal Nehru Medical College Aligarh*, was reported to be effective against several pathogens found in women with vulvovaginal vaginitis. The "BASANT" is made up of 95 percent pure Curcumin exctrated from *Curcuma longa*, refined extracts of *Emblicaofficinalis*, Neem (*Azadirachta indica*) leaves, and Aloe vera (*Aloe barbadensis*) [50].

Feminine Wash for candidiasis with the Extraction of *Piper betle* provided the greatest patient compliance with the least amount of negative effects [51]. At a concentration of 62.5 mg/ml, the designed product proved effective as a topical drug delivery system.

Over the decades, *Tamarindusindica* is using a commercialized traditional medicine as an anti-inflammatory and analgesic drug because it possess the potential to suppress the diversity of biological expressions including COX-2 (cyclooxygenase-2) expression, iNOS (inducible nitric oxide synthase) biosynthesis, 5-lipoxygenase biosynthesis, and α-TNF (tumor necrosis factor-α)[52]. *T. indica's* commercially valued therapeutic properties are also linked to the presence of phytochemicals in the plant's many components, including flavonoids, alkaloids, tannins, phenols, triterpenoids, fatty acids, saponins, and steroids [53,54]. In a research on *T. indica* against bacterial urinary tract infection, it was reported to have the maximum inhibitory activity against *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella sp*., and *Enterococcus sp*. compared to the reference standard [55]. Recent studies revealed that tamarind pulp and leaf extract may be utilised as an efficient agent against *Pseudomonas putida*, *Enterobactergergovia, Escherichia coli, Bacillus subtilis, Hafniaalvei, Salmonella typhi*, and *Staphylococcus aureus* [56,57].

In addition, *Calendula officinalis* is an aromatic plant that is used in the traditional system of medicine for tissue repair, lesions, herpes, scars, skin irritation, and blood cleansing. Particularly in Ayurveda, the solution or tincture of calendula is

applied medically to cure dermatitis and fungal infections, reduce inflammation, regulate bleeding, and heal inflamed tissue [58]. Many pharmaceutically active compounds, including carotenoids, flavonoids, glycosides, steroids, glycolipids quinines, volatile oils and amino acids are included in Calendula's formulations. Isorhamnetin, rutin, and quercetinglucoside have been identified as new medically active chemical ingredients by advanced technical investigation [59]. Calendula is used to treat a variety of ailments, including skin inflammations, open wounds, and laceration wounds with bleeding. It may also be given for gastrointestinal spasms, as well as menstrual irregularities and the mucosa of the duodenum and intestines. The plant has promising antibacterial effects against *Bacillus subtulis, Staphylococcus aureus, Staphylococcus anrens, Escherichia coli, Klebsiella pneumonia, Salmonella typhae, Candida albicans*, and *Aspergillusniger* [60-63]. Similarly, in the ethno medical system, *W. somnifera* is a novel plant employed as an anti-oxidant, tonic, sensual, anti-aging product, anti-hyperglycemic agent, anti-inflammatory agent, antiseptic, musculotropic action, immunoregulatory function, and antibacterial agent [64-66]. Phytochemicals from *W. somnifera* have been shown to be effective against a wide range of infections, including *E. aerogens*, *B. subtilis, K. pneumoniae, Raoultellaplanticola, S. aureus, B. anthracis, S. typhi, P. aeruginosa,A. fumigates, C. albicans, C. vaginitis*, and *B. dermatidis* [67-70].

The above-cited research suggests that conventional cosmetic and herbal feminine hygiene products may not be fully safe and may have some negative consequences. In order to address this issue at the commercial level, prospective natural photochemical components like *Withanias omanifera*, *Calendula Officinalis*, and *Tamarindusi ndica* may be suitable possibilities.

2.1 Importance of the Proposed Work in the Context of Current Status

Due to regular usage of available synthetic intimate hygiene feminine washes, pH of Vagina is very much reduced by products that prevents the infections but kill all the healthy bacteria like lactobacillus. These Healthy bacteria maintain the vaginal lubrication, natural pH& sensory of vagina. As well, after long period, some more dermal aggressive anaerobic pathogens including Helicobacter *pylori*, replace the lactobacilli and generate over production of over acidic or dry medium which cause burning, itching, redness and sometime vaginal ulcers *which may ultimately lead to reproductive cancers*. It is usual in post menopause bearing women.

2.1.1 Novelty of proposed work

- *Therefore, the development of Herbal Germicide-Aided Natural Solution for Women's Hygiene is proposed to be investigated in this study.*
- *The developed product will stand in defence mechanism against vaginal pathogens itself.*
- *Furthermore, it will help to support to grow natural lactobacillus in vaginal system and to maintain there effective pH with therapeutic effectiveness and healthy conditions.*
- *In addition, based on the findings, the proposed study may provide a straightforward solution to the problem of Vaginal Vaginitis.*
- *Besides, depending on the outcome, there is definite product development possibility of an efficient herbal vaginal wash, at least at the laboratory scale at the first stage.*
- *Once a successful formulation is developed and its efficacy tested beyond doubt, the possibility of patent should definitely pop-up.*

2.1.2 Implications/significance of proposed work

Lab-scale effort might provide significant results in the form of successful herbal bioactive compounds extractions. These results/ technology concepts may be scaled up to provide an economically attractive herbal Vagainal wash for the direct superficial application on vaginal dermis. This will enable for the implementation of novel and non-toxic, chemical free Vagainal infection meditation techniques.

2.2 Objectives

2.2.1 The primary objective

Development of Herbal Germicide-Aided Natural Solution for Women's Hygiene

2.2.2 The secondary objective

- To extract crude extracts from the plants *Tamarindus indica, Withanias omnifera*, and *Calendula officinalis.*

- To identify and purify the bioactive compounds from extracted extracts.
- To evaluate the antimicrobial activity of purified bioactive compounds against Vaginal Microflora.

3. METHODOLOGY

3.1 Primary

3.1.1 Estimation of antimicrobial efficacy

The antimicrobial efficacy of purified bioactive compounds from *Tamarindusindica, Withania somnifera*, and *Calendula officinalis*plants have estimated by disc diffusion method as deemed appropriate (e.g., Gould and Bowie, 1952; CLSI, 2015) against Vaginal Microflora. In due respect, initially invitro study will be designed where Vaginal microflora have categorized as most dominant pathogenic strains of *Staphylococcus aureus; Streptococcus bovis; Staphylococcus epidermidis; Helicobacter pylori; Candida albicans* and *Lactobacillus gasseri* species [70]. All above selected strains have procured from national culture collection sources. The Minimum inhibitory concentration have also determined by micro dilution method for active compounds extracted from selected plants against selected test microrganisms [71].

3.2 Pre-Primary

3.2.1 Plant material collection

Various plant parts (leaves, barks and flowers) of *Tamarindus indica, Withanias omnifera*, and *Calendula officinalis* have separately collected, dried, powdered and used for further studies (Crude extraction and antimicrobial study). Before processing of drying & powdering, the plant specimen identification have confirmed by comparing with those available in the Herbarium, Department of Botany, University of Rajasthan, Jaipur or those available in reference sources *available in India*.

3.2.2 Extraction of crude

Crude Extracts will be extracted from various dried plant parts (leaves and flowers) by hydro distillation method using Clevenger or Soxhlet extractor apparatus [72].

3.2.3 Characterization of crude extract

Crude Extracts will be Characterized qualitative and quantitative methods by following Gas Chromatography & Gas Chromatography Mass Spectroscopy techniques.

3.2.4 Separation the bioactive compounds from extracted extracts

Different Bioactive compounds from Crude extracts have separated by methods as deemed appropriate. In the present study, Crude fractions have performed with the help of available common practices including HP-TLC, Analytical HPLC, and UHPLCor solid-liquid extraction by column chromatography methods. In this objective, solvent systems have also planned based on polarity of bioactive compounds. The separated compound have dried using rotatory evaporator to form pure compounds which will be utilized for the determination of structure and biological activity.

3.2.5 Purification and quantification of the bioactive compounds from extracted extracts

Chromatographic analysis have performed on an HPLC system, equipped with a diode array detector (DAD) and Flash Chromatography system. Both systems have controlled by chromatographic software. Chemical Composition and Identification of purified compound analysis of bioactive compounds have performed using NMR System.

3.3 Post-Primary

3.3.1 Designing of herbal germicide-aided natural product using potential bioactive compounds and clinical validation for women's hygiene

- After *in vitro* study of *first phase* of antimicrobial efficacy of purified bioactive compounds from selected plants against vaginal microflora,
- Attempt have made in the second stage to formulate hygiene product from active compounds.
- The processed hygiene products have further evaluated for clinical validation and product have designed for future usage by the mass populations.
- The designed product have characterized qualitative and quantitative by following the Gas Chromatography (GC) and Gas Chromatography Mass Spectroscopy (GCMS) techniques.

4. RESULTS AND DISCUSSION

- We found that herbal germicide prepared by Various plant parts (leaves, barks and flowers) of *Tamarindusindica, Withania somnifera*, and *Calendula officinalis as active as synthetic germicide.*
- Continue use of synthetic viginal wash can causes many type of infections, skin problems and feeling uncomfortable but this natural herbal v-wash never creat such type of problems it can be use daily without any doctor's prescription infact it maintains our vaginal health issues and provides them better life style.

5. CONCLUSION

Synthetic intimate wash is a solution it is commonly used for the diagnosis or treatment of dryness, itching, irritation. It has some side effects such as dryness, feeling of warmth, itching, allergic reaction. So we are suggesting here to use herbal intimate hygiene product instead synthetic hygiene product it is also as effective as synthetic products**.**

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Lamont RF, Sobel JD, Akins RA, Hassan SS, Chaiworapongsa T, Kusanovic JP et al. The vaginal microbiome: new information about genital tract flora using molecular based techniques. BJOG. 2011;118(5):533-49.
2. Lin YP, Chen WC, Cheng CM, Shen CJ. Vaginal pH value for clinical diagnosis and treatment of common vaginitis. Diagnostics (Basel). 2021;11(11).
3. Gajdács M, Dóczi I, Ábrók M, Lázár A, Burián K. Epidemiology of candiduria and Candida urinary tract infections in inpatients and outpatients: results from a 10-year retrospective survey. Cent Eur J Urol. 2019;72(2):209-14.
4. Bitew A, Mengist A, Belew H, Aschale Y, Reta A. The prevalence, antibiotic resistance pattern, and associated factors of bacterial vaginosis among women of the reproductive age group from Felege Hiwot referral hospital, Ethiopia. Infect Drug Resist. 2021;14:2685-96.
5. DRM, GK, KJ, DD, TS, Dinesh P. Evaluation of Salivary Flow Rate, PH and buffer in Pre, Post & Post menopausal women on HRT. J Clin Diagn Res. 2014; 8(2):233-6.
6. Panda S, Das A, Singh AS, Pala S. Vaginal pH: A marker for menopause. J Mid Life Health. 2014;5(1):34-7.
7. Vieira AT, Castelo PM, Ribeiro DA, Ferreira CM. Influence of oral and gut microbiota in the health of menopausal women. Front Microbiol. 2017;8(SEP).
8. Ling Z, Kong J, Liu F, Zhu H, Chen X, Wang Y et al. Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. BMC Genomics. 2010;11(1):488.
9. Paladine HL, Desai UA. Vaginitis: Diagnosis and treatment. Am Fam Physician. 2018;97(5):321-9.
10. Aguirre-Quiñonero A, Castillo-Sedano, de IS, Calvo-Muro F, Canut-Blasco A. Accuracy of the BD MAXTM vaginal panel in the diagnosis of infectious vaginitis. Eur J Clin Microbiol Infect Dis. 2019;38(5): 877-82.
11. Ahangari F, Farshbaf-Khalili A, Javadzadeh Y, Adibpour M, Sadeghzadeh Oskouei B. Comparing the effectiveness of Salvia officinalis, clotrimazole and their combination on vulvovaginal candidiasis: A randomized, Controlled; 2019.
12. Buggio L, Somigliana E, Borghi A, Vercellini P. Probiotics and vaginal microecology: Fact or fancy? BMC Womens Health. 2019;19(1):25.
13. Swidsinski A, Guschin A, Tang Q, Dörffel Y, Verstraelen H, Tertychnyy A et al. Vulvovaginal candidiasis: histologic lesions are primarily polymicrobial and invasive and do not contain biofilms. Am J Obstet Gynecol. 2019;220(1):91.e1-8.
14. Łaniewski P, Ilhan ZE, Herbst-Kralovetz MM. The microbiome and gynaecological cancer development, prevention and therapy. Nat Rev Urol. 2020;17(4):232-50.
15. Sim M, Logan S, Goh LH. Vaginal discharge: Evaluation and management in primary care. Singapore Med J. 2020;61(6):297-301.
16. Hani U, Bhat RS, Shivakumar HG. Formulation design and evaluation of metronidazole microspheres in a bioadhesive gel for local therapy of vaginal candidiasis. Lat Am J Pharm. 2011;30(1).
17. Trivedi D. Isolation Characterization and Assessment of antimicrobial potential of

Urino Vaginal microorganisms and their applications in the development of micribicides [Ph. D. thesis]. Nirma University; 2014.
18. Gaziano R, Sabbatini S, Roselletti E, Perito S, Monari C. *Saccharomyces cerevisiae*-based probiotics as novel antimicrobial agents to prevent and treat vaginal infections. Front Microbiol. 2020; 11:718.
19. Coudray MS, Madhivanan P. Bacterial vaginosis—A brief synopsis of the literature. Eur J Obstet Gynecol Reprod Biol. 2020;245:143-8.
20. Sherrard J, Wilson J, Donders G, Mendling W, Jensen JS. 2018 European (IUSTI/WHO) international union Silva; 2018. Silva D, Ferreira MS, Sousa-Lobo JM, Cruz MT, Almeida IF. Anti-inflammatory activity of *Calendula officinalis* L. Flower extract. Cosmetics. 2021;8(2):31.
21. Kenyon CR, Osbak K. Recent progress in understanding the epidemiology of bacterial vaginosis. Curr Opin Obstet Gynecol. 2014;26(6):448-54.
22. Tidbury FD, Langhart A, Weidlinger S, Stute P. Nonantibiotic treatment of bacterial vaginosis—a systematic review. Arch Gynecol Obstet. 2021;303(1): 37-45.
23. Klebanoff MA, Nansel TR, Brotman RM, Zhang J, Yu KF, Schwebke JR et al. Personal hygienic behaviors and bacterial vaginosis. Sex Transm Dis. 2010;37(2): 94-9.
24. Fashemi B, Delaney ML, Onderdonk AB, Fichorova RN. Effects of feminine hygiene products on the vaginal mucosal biome. Microb Ecol Health Dis. 2013;24(0).
25. Chen Y, Bruning E, Rubino J, Eder SE. Role of female intimate hygiene in vulvovaginal health: Global hygiene practices and product usage. Womens Health (Lond). 2017;13(3):58-67.
26. Chow WH, Daling JR, Weiss NS, Moore DE, Soderstrom R. Vaginal douching as a potential risk factor for tubal ectopic pregnancy. Am J Obstet Gynecol. 1985;153(7):727-9.
27. Martino JL, Vermund SH. Vaginal douching: evidence for risks or benefits to women's health. Epidemiol Rev. 2002; 24(2):109-24.
28. Luong ML, Libman M, Dahhou M, Chen MF, Kahn SR, Goulet L et al. Vaginal douching, bacterial vaginosis, and spontaneous preterm birth. J Obstet Gynaecol Can. 2010;32(4):313-20.
29. Yanikkerem E, Yasayan A. Vaginal douching practice: frequency, associated factors and relationship with vulvovaginal symptoms. J Pak Med Assoc. 2016;66 (4):387-92.
30. Jenkins AL, Crann SE, Money DM, O'Doherty KC. 'Clean and Fresh': Understanding women's use of vaginal hygiene products. Sex Roles. 2018;78(9-10):697-709.
31. Gabriel IM, Vitonis AF, Welch WR, Titus L, Cramer DW. Douching, talc use, and risk for ovarian cancer and conditions related to genital tract inflammation. Cancer Epidemiol Biomarkers Prev. 2019;28 (11):1835-44.
32. Gondwe T, Ness R, Totten PA, Astete S, Tang G, Gold MA et al. Novel bacterial vaginosis-associated organisms mediate the relationship between vaginal douching and pelvic inflammatory disease. Sex Transm Infect. 2020;96(6):439-44.
33. Dimitriadi D. *Helicobacter pylori*: A sexually transmitted bacterium? Cent Eur J Urol. 2014;67(4):407-9.
34. Sánchez-Alonzo K, Matamala-Valdés L, Parra-Sepúlveda C, Bernasconi H, Campos VL, Smith CT et al. Intracellular presence of Helicobacter pylori and its virulence-associated genotypes within the vaginal yeast of term pregnant women. Microorganisms. 2021;9(1):131.
35. Amiri FN, Rooshan MH, Ahmady MH, Soliamani MJ. Hygiene practices and sexual activity associated with urinary tract infection in pregnant women. East Mediterr Health J. 2009;15(1):104-10.
36. Rosenblatt KA, Weiss NS, Cushing-Haugen KL, Wicklund KG, Rossing MA. Genital powder exposure and the risk of epithelial ovarian cancer. Cancer Causes Control. 2011;22(5):737-42.
37. Ahmed OI, Nourein IH, Elbadri M, Abdelgadir AE, Ayed I, Shalayel M. The most common causes of cervico-vaginal infections among Sudanese women complaining of vaginal discharge. Int J Clin Med Microbiol. 2016;1(1).
38. Schildkraut JM, Abbott SE, Alberg AJ, Bandera EV, Barnholtz-Sloan JS, Bondy ML et al. Association between body powder use and ovarian cancer: The African American cancer epidemiology study (AACES). Cancer Epidemiol Biomarkers Prev. 2016;25(10):1411-7.

39. Jessima S. Prevalence of urinary tract infection among school going adolescent girls in rural part of Chennai. Int Arch Integr Med. 2019;6(3).
40. Mohiuddin AK. Lifestyle issues and prevention of recurrent UTIs. Mathews J Urol Nephrol. 2019;3(1).
41. Robert M. Second-degree burn sustained after vaginal steaming. J Obstet Gynaecol Can. 2019;41(6):838-9.
42. Freitas AC, Bocking A, Hill JE, Money DM, VOGUE Research Group. Increased richness and diversity of the vaginal microbiota and spontaneous preterm birth. Microbiome. 2018;6(1):117.
43. Onderdonk AB, Delaney ML, Fichorova RN. The human microbiome during bacterial vaginosis. Clin Microbiol Rev. 2016;29(2):223-38.
44. Yudin MH, Money DM. No. 211-screening and management of bacterial vaginosis in pregnancy. J Obstet Gynaecol Can. 2017;39(8):e184-91.
45. Jenkins A, Money D, O'Doherty KC. Is the vaginal cleansing product industry causing harm to women? Expert Rev Anti-Infect Ther. 2021;19(3):267-9
46. Ozen B, Baser M. Vaginal candidiasis infection treated using apple cider vinegar: A case report. Altern Ther Health Med. 2019;25(5).
47. Nakagawa S, Hillebrand GG, Nunez G. *Rosmarinus officinalis* L. (Rosemary) extracts containing carnosic acid and carnosol are potent quorum sensing inhibitors of Staphylococcus aureus virulence. Antibiotics (Basel). 2020;9(4): 149.
48. Ezz Eldin HM, Badawy AF. In vitro anti-*Trichomonas vaginalis* activity of *Pistacia lentiscus* mastic and *Ocimum basilicum* essential oil. J Parasit Dis. 2015;39(3):465-73.
49. Talwar GP, Sharma R, Singh S, Das BC, Bharti AC, Sharma K et al. BASANT, a polyherbal safe microbicide eliminates HPV-16 in women with early cervical intraepithelial lesions. J Cancer Ther. 2015;06(14):1163-6.
50. Naimat F, Annissa N, Zaki AM, Ridzuan PM. Formulation and evaluation of feminine wash for candidiasis with the extraction of piper Betle as antifungal effect. Ann Rom Soc Cell Biol. 2021;25.
51. Komakech R, Kim YG, Matsabisa GM, Kang Y. Anti-inflammatory and analgesic potential of *Tamarindus indica* Linn. (Fabaceae): A narrative review. Integr Med Res. 2019;8(3):181-6.
52. Khanzada SK, Shaikh W, Sofia S, Kazi TG, Usmanghani K, Kabir A et al. Chemical constituents of *Tamarindus indica* L. medicinal plant in Sindh. Pak J Bot. 2008;40(6).
53. Abdelrahman GH, Mariod AA. Tamarindus indica: phytochemical Constituents, Bioactive compounds and Traditional and Medicinal Uses. In: Wild fruits: composition, nutritional value and products. Cham: Springer International Publishing. 2019;229-38.
54. Biswas K, Sinha SN. Antimicrobial Activity of *Tamarindus indica* L. against bacteria causing urinary tract infection. J Bio Sci. 2015;23:47-55.
55. Daniyan SY, Muhammad HB. Evaluation of the antimicrobial activities and phytochemical properties of extracts of *Tamaridus indica* against some diseases causing bacteria. Afr J Biotechnol. 2008;7(14).
56. Adeniyi O, Olaifa F, Emikpe B, Ogunbanwo S. Phytochemical components and antibacterial activity of *Tamarindus indica* Linn. Extracts against some pathogens. Biotechnol J Int. 2017;17(2): 1-9.
57. Ashwlayan VD, Kumar A, Verma M, Garg VK, Gupta S. Therapeutic potential of *Calendula officinalis*. Pharm Pharmacol Int J. 2018;6(2).
58. Arora D, Rani A, Sharma A. A review on phytochemistry and ethnopharmacological aspects of genus Calendula. Pharmacogn Rev. 2013;7(14):179-87.
59. Efstratiou E, Hussain AI, Nigam PS, Moore JE, Ayub MA, Rao JR. Antimicrobial activity of *Calendula officinalis* petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens. Complement Ther Clin Pract. 2012;18(3):173-6.
60. Abudunia AM, Ansar M, Taoufik J, Ramli Y, Essassi EM, Ibrahimi A et al. Evaluation of antibacterial activity of extracts from *Calendula aventis* flowers. J Chem Pharm Res. 2017;6(8).
61. Vora J, Srivastava A, Modi H. Antibacterial and antioxidant strategies for acne treatment through plant extracts. Inform Med Unlocked. 2018;13:128-32.
62. Rojas Bedoya LC, Gómez López K, Marín Pareja N. Extraction of metabolites from *Calendula officinalis* and evaluation of their

colorant and antibacterial capacity. Rev Colomb Biotecnol. 2020;22(1):60-9.
63. Thakur RS, Puri HS, Hussain A. Major medicinal plants of India. India: Central Institute of Medicinal and Aromatic Plants. Lucknow; 1989.
64. Dharajiya D, Patel P, Patel M, Moitra N. In vitro antimicrobial activity and qualitative phytochemical analysis of *Withania somnifera* (L.) dunal extracts. Int J Pharm Sci Rev Res. 2014;27(2).
65. Saleem S, Muhammad G, Hussain MA, Altaf M, Bukhari SNA. *Withania somnifera* L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. Iran J Basic Med Sci. 2020;23(12):1501-26.
66. Kambizi L, Afolayan AJ. Extracts from Aloe ferox and Withania somnifera inhibit *Candida albicans* and *Neisseria gonorrhoeae*. Afr J Biotechnol. 2008;7(1).
67. Panchal P, Singh K. Antimicrobial activity of *Withania sominfera* and *Calotropis procera* on pathogenic strains. Int J Curr Pharm Rev Res. 2015;7(4).
68. Khanchandani N, Shah P, Kalwani T, Ardeshna A, Dharajiya D. Antibacterial and antifungal Activity of Ashwagandha (*Withania somnifera* L.): A review. J Drug Deliv Ther. 2019;9(5-s):154-61.
69. Chen X, Lu Y, Chen T, Li R. The female vaginal microbiome in health and bacterial vaginosis. Front Cell Infect Microbiol. 2021;11:631972.
70. Institute C, LS. M02-A12: Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Twelfth Edition. Clinical and Laboratory Standards Institute. 2015;35 (M02-A12).
71. Luque de Castro MD, García-Ayuso LE. Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. Anal Chim Acta. 1998;369(1-2):1-10.
72. Abd Aziz NA, Hasham R, Sarmidi MR, Suhaimi SH, Idris MKH. A review on extraction techniques and therapeutic value of polar bioactives from Asian medicinal herbs: case study on *Orthosiphon aristatus*, *Eurycoma longifolia* and *Andrographis paniculata*. Saudi Pharm J. 2021;29(2):143-65.

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