Development of Herbal Germicide-Aided Natural Solution for Women's Hygiene without any side and adverse effect in laboratory

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 v wash .This article provides context on the herbal germicide-aided natural solution for women's hygiene. For that purpose we use Withania Somnifera plant and 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.

Keywords : Female intimate hygiene,vaginal wash,natural ,herbal ,germicide microbiota

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.*

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, (Lamont et al., 2011; Lin et al., 2021). 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 (Gajdács et al., 2019; Bitew et al., 2021). 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 (Mahesh et al., 2014; Panda et al., 2014; Vieira et al., 2017). 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 (Ling et al., 2010). *Atopobiumvaginae, Gardnerellavaginalis, Megasphaeraspp, Staphylococcus aureus, Streptococcus bovis, Staphylococcus epidermidis, Helicobacter pylori, Mycoplasma hominis, Ureaplasmaurealyticum*, *Trichomonasvaginalis*, *Candida albicans*, etc. are some of the microbes that cause infections with bad-smelling discharge at Female genital system (Paladine et al., 2018; Aguirre-Quinonero et al., 2019; Ahangari et al., 2019; Buggio et al., 2019; Swidsinski et al., 2019; Laniewski et al., 2020; Sim et al., 2020; Bitew et al., 2021).

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 (Hani et al., 2011). 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 (Trivedi, 2014). 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 (Yadav, 2018; Gaziano et al., 2020). Most often, antibiotics metronidazole and clindamycin, given orally or vaginally, or both, are suggested solely for women who are exhibiting symptoms (Coudray and Madhivanan, 2020; Sherrard et al., 2018). However, it was reported in a study that after successful antibiotic delivery, reinfection developed due to biofilm formation on the vaginal dermis (Kenyon and Osbak, 2014; Tidbury et al., 2021).

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 (Klebanoff et al., 2010; Fashemi et al., 2013; Chen et al., 2017).

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 (Chow et al., 1989; Martino and Vermund, 2002; Cook et al., 2009; Luong et al., 2010; Yanikkerem and Yasayan, 2016; Jenkins et al., 2018; Gabriel et al., 2019; Gondwe et al., 2020). 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 (Dimitriadi, 2014; Sánchez-Alonzo et al., 2021). Similarly, another research (Amiri et al. 2009) 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 (Rosenblatt et al., 2011; Feshmi et al., 2013; Ahmed et al., 2016; Schildkraut et al., 2016; Jessima, 2019; Mohiuddin, 2019; Robert, 2019). According to research, intra-vaginal cleaning agents alter the microbiome by eliminating beneficial bacteria and mucus from the vaginal canal (Freitas et al., 2018). 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 (Onderdonk et al., 2016).

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 (Yudin and Money,2017; OWH-US,2022; Jenkins et al., 2021). 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, 2017 demonstrated that apple vinegar was a very effective antibiotic and disinfectant against vaginal candidiasis infection. In a research conducted by Nakagawa et al. (2020), rosemary extracts were shown to be the most efficient against S. aureus recovered from clinical isolates. In addition, research (Eldin and Badawy, 2015) indicated that phytotherapeutic compounds of *Pistacialentiscus mastic* and *Ocimumbasilicum* efficiently demonstrate anti-*Trichomonasvaginalis* 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 (*Azadirachtaindica*) leaves, and Aloe vera (*Aloe barbadensis*)(Talwar et al., 2016).

Feminine Wash for candidiasis with the Extraction of *Piper betle* provided the greatest patient compliance with the least amount of negative effects (Naimat et al., 2021). 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)(Komakech et al., 2019). *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 (Khanzada et al., 2008;Abdelrahman and Mariod, 2019). 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 (Biswas and Sinha, 2015). 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* (Daniyan and Muhammad,2008; Adeniyi et al. 2017).

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 (Ashwlayan et al., 2018). 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 (Arora et al., 2013). 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* (Efstratiou et al., 2012; Abudunia et al., 2017; Ashwlayan et al., 2018; Vora et al., 2018; Rojas-Bedoya et al., 2020; Silva et al., 2021). 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 (Thakur et al., 1989; Jain et al., 2012; Dharajiya et al., 2014; Saleem et al., 2020). 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*(Kambiz and Afolayan, 2008; Bisht and Rawat, 2014; Panchal and Singh, 2015; Khanchandani et al., 2019).

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 *Withaniasomanifera*, *Calendula Officinalis*, and *Tamarindusindica* may be suitable possibilities.

2.1. Importance of the proposed work in the context of current status:

Due to regular usage of available 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.

- ✧ *Therefore, the development of Herbal Germicide-Aided Natural Solution for Women's Hygiene is proposed to be investigated in this study. This is the first unique novelty of the proposed work.*

- ✧ *The developed product will stand in defense mechanism against vaginal pathogens itself. This is the second unique novelty of the proposed work.*
- ✧ *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. This is the third unique novelty of the proposed work.*
- ✧ *In addition, based on the findings, the proposed study may provide a straightforward solution to the problem of Vaginal Vaginitis. This is the fourth unique novelty of the proposed work.*
- ✧ *Besides, depending on the outcome, there is definite product development possibility of an efficient herbal Vagainal wash, at least at the laboratory scale at the first stage. This is the fifth unique novelty of the proposed work.*
- ✧ *Once a successful formulation is developed and its efficacy tested beyond doubt, the possibility of patent should definitely pop-up. This is the sixth unique novelty of the proposed work.*

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.

Objectives:

The Major objective:

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

The Sub-Major objective:

- To extract crude extracts from the plants *Tamarindusindica*, *Withaniasomnifera*, 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.

Methodology:

Primary:

- **Estimation of Antimicrobial efficacy:**

  The antimicrobial efficacy of purified bioactive compounds from *Tamarindusindica, Withaniasomnifera*, 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 (Chen et al., 2021). 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 (CLSI, 2015).

Pre-Primary

- **Plant Material Collection:**

  Various plant parts (leaves, barks and flowers) of *Tamarindusindica, Withaniasomnifera*, 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*.

- **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 (Clevenger et al., 1928).

- **Characterization of Crude Extract:**

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

- **Separation the bioactive compounds from extracted extracts:**

  Different Bioactive compounds from Crude extracts have separated by methods as deemed appropriate (e.g., Elamin, 2020;Aziz et al., 2021; Fongang et al., 2021). 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.

- **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.

Post-Primary:

- **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.

❖ Results and discussion

❖ We found that herbal germicide prepared by Various plant parts (leaves, barks and flowers) of *Tamarindusindica*, *Withaniasomnifera*, 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

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