



Formulation And Evaluation of Herbal Cream Containing Azadirachta Indica for Antifungal, Antibacterial Activity

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ABSTRACT

Development and Evaluation of a Polyherbal Cream Containing Ajwain, Betel Leaf, Neem Oil, and Tea Tree Oil for Relief of Vaginal Irritation and Associated Yeast Infections. Vaginal irritation and yeast infections are common gynecological issues affecting women's quality of life. Current treatments often have limitations, including antibiotic resistance and side effects.

Our objective is to formulate and evaluate a polyherbal cream containing Ajwain

(Trachyspermum ammi), Betel Leaf (Piper betle), Neem Oil (Azadirachta indica), and Tea Tree Oil (Melaleuca alternifolia) for relief of vaginal irritation, itching, and soreness associated with yeast infections.

The polyherbal cream is a safe and effective treatment for vaginal irritation and associated yeast infections, offering a natural alternative to conventional treatment.

Keywords: Vaginal infection; Yeast infection; Gynecological; Ajwain; Piper betle; Azadirachta indica; Polyherbal

INTRODUCTION

As women age, changes occur in the vagina, the female reproductive organ. It has several functions during puberty, menstruation, pregnancy, and menopause in response to hormonal changes. The vagina is vital to the female reproductive system and sexual pleasure

Infections or inflammations that alter the physiological milieu of the vaginal mucosa are common. Worldwide, vaginal candidiasis is a prevalent issue among sexually active women, with up to 75% of all women experiencing at least one bout of the infection in their lifetime. More than 80% of vaginal candidiasis infections are caused by *Candida albicans*, making it the most significant source of the infection [1].

Though they have adverse effects, some of the medications used to treat vaginal candidiasis are Metronidazole, Ketoconazole, Clotrimazole, Fluconazole, Itraconazole, Secnidazole, Mystatin, Progesterone, Acriflavine, etc [2-5]. An intriguing and mainly unexplored source for the production and development of possibly novel antibacterial drugs is found in traditional medicines [6-10]. The first and most important stage in achieving this goal is the screening of plants in popular medicine to offer practical solutions for a variety of illnesses that are stubborn and incurable with conventional medical systems. These are becoming more and more popular because to a number of benefits, including fewer side effects, improved patient tolerance, lower costs, and greater acceptability because of their lengthy history of usage. In addition to correcting the underlying cause of the condition and normalising physiological function, the

medicinal benefits of plants also seem to make medication resistance less likely to develop [9,10].

Anatomic overview

The vagina is a vital organ of the reproductive system and plays a significant part in reproduction [11].

The wall structure

Stretching approximately 7.5 cm from the uterus to the vestibule of the external genitalia, it is a robust muscular canal (Figure 1A). The vaginal wall is folded crosswise for adequate elasticity (Figure 1.1 B). The vagina lies between the urethra, bladder, and rectum. It differs greatly from the intestinal wall in both architecture and function is not as hard as the gut, but it also does not have peristaltic action.

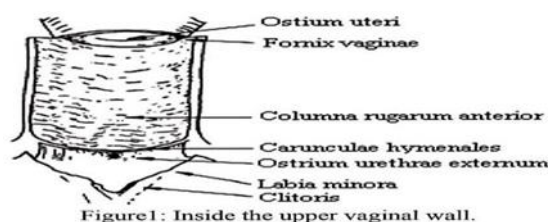


Figure1: Inside the upper vaginal wall.

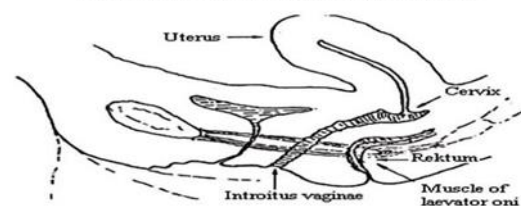


Figure1: Graphical description of the vagina

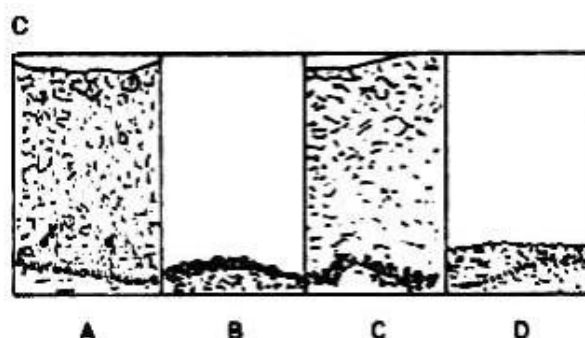


Figure 1: (A) Inside the upper vaginal wall (B) Graphical description of the vagina located between rectum and bladder

(C) Comparison of epithelial thickness of the vaginal tissue, A-newborn, B-child, C-adult, D-menopause.

Three layers make up the vaginal wall: the tunica adventitia, the muscular coat, and the epithelial layer. It is thought that a cell turnover of roughly 10–15 layers occur every 7 days.² The squamous epithelium is stratified and noncornified. Age determines the thickness. The vaginal epithelium thickens with hormonal activity, achieves its maximum glycogen content after ovulation, and is at its thickest during the proliferative period. Differential cytology of the vaginal epithelium can be utilised to determine the cycle stages, even though the endometrium exhibits more

significant cyclic changes than the vaginal epithelium. It is evident that the thickness of the epithelium varies according to the various life stages, including infant, child, adult, and menopause (Figure 1C) [11]

Arteries and veins

The vaginal branch of the uterine artery provides the vagina with its primary blood supply

When developing and accessing vaginal delivery systems, it is important to take into account the vagina's distinct microbiota, pH, and cyclic fluctuations [11].

Epithelium

The vagina is protected from infection by the vaginal fluids, cervical mucus, and local bacterial flora in addition to the physical barrier provided by the vaginal epithelium, which is 25 layers deep and contains estragon. Because of the stratified squamous epithelium's continuous shedding, organisms find it challenging to enter or reach the capillary bed or basement membrane [12,13].

Micro flora

The amount of glycogen in the epithelial cells, glucose, pH, hormones, trauma during sexual activity, birth control method, age, antimicrobial treatment, and delivery are some of the variables that affect the vaginal ecology. The most common organism in the vaginal environment, *Lactobacillus* (Do' Delrin's bacilli), along with numerous other facultative and obligatory aerobes and anaerobes, make up the dynamic vaginal flora. It was discovered that vaginal biopsies were ideal for illustrating the dispersion of vaginal mucosa. Throughout the cycle, the superficial vaginal epithelium's glycogen content tended to rise before declining in the late premenstrual period. This was in accordance with the cycle's excretion of oestrogen.

The vaginal glycogen content permanently declines as oestrogen synthesis declines during the premenopausal and subsequent menopausal phases. The creatures that are acidophilic are no longer in control. Numerous microorganisms are present throughout this time, but none of them predominates [11,13].

Vaginal pH

Lactobacilli, which make up the majority of the normal microbiota, generate enough lactic acid to raise the pH of vaginal secretions to 3.5 to 4.5. The *lactobacilli*, which turn glycogen from exfoliated epithelial cells into lactic acid, preserve this value. Age, menstrual cycle phases, illnesses, and sexual excitement all affect pH. The vagina of the majority of women has a pH gradient. Semen and secretions from the uterus, cervical region, and menstruation serve as alkalisating agents and raise the P. 5 The pH is significant for drug delivery systems and influences the amount of medication absorption. To rule out a breakdown of the epithelial barrier, the design of such a drug delivery system should be proportionate to the volume of vaginal fluid [11,12].

Cyclic changes

The thickness of the epithelial cell layer, the breadth of intercellular channels, pH, and secretions are all affected by changes in hormone levels, particularly oestrogen, that occur during the menstrual cycle. The fluctuations in enzyme activity

(endopeptidases and aminopeptidases) with hormonal changes further complicate the difficulty of establishing constant medication administration [11].

Vaginal mucoadhesive drug delivery system

The majority of traditional vaginal formulations have a number of drawbacks, including inadequate retention to the vaginal epithelium, leaking, and messiness, which results in low patient compliance. Mucoadhesive vaginal medication delivery is being promoted as a solution to these problems. The situation in which two materials—at least one of which is biological in nature—are kept together for prolonged periods of time by interfacial forces is known as mucoadhesion. The phenomenon is sometimes called mucoadhesion if the attachment is caused by a mucus layer. For mucoadhesive drug delivery systems, to maintain systems for treating primarily local (but some systemic) illnesses, or for use in contraception, the vaginal route seems to be especially suitable. One of the most researched mucoadhesive formulations for vaginal medication delivery is gel [14].

Pathophysiology

The normal flora of the vagina is maintained by a complex and delicate balance of microorganisms. Yeast, corynebacteria, and *lactobacilli* are important organisms. The vagina of prepubertal girls, pubertal adolescents, and adult women can all produce both aerobic and anaerobic bacteria. Women who are premenopausal or postmenarchal typically have a vaginal pH between 3.8 and 4.2. Usually, this pH prevents harmful organisms from growing. The vaginal flora may alter when the usual pH balance is disturbed, which may result in an overabundance of pathogens. Numerous factors, including as stress, sexual activity, antibiotics, feminine hygiene products, contraception, and vaginal drugs, might change the vaginal environment. Vaginitis symptoms can be brought on by an excess of viruses or harmful microorganisms, which are usually bacteria. Another important consideration may be chemical irritation [15].

Atrophic vaginitis, which results in irregular bleeding, dryness, itching, and dyspareunia, is linked to hypoestrogenism. A situation where oestrogen levels are lower could lead to a different risk of infection. Kenyon and Coalbunkers discovered evidence indicating women who have a male sexual partner who is also having sex with other partners are at a higher risk of contracting bacterial vaginosis, based on data from 11 different nations. The physiology and morphology of the vagina are influenced by the patient's age. Compared to women and teenagers in their pubertal and post pubertal years, prepubescent children have a higher

alkaline vaginal pH. The normal vaginal flora is similar to that of postmenopausal women (e.g., gram-positive cocci and anaerobic gram- negatives are more common), the labia are thin with a thin hymen, and the vaginal mucosa is squamous epithelium. Moreover, there are no mucous glands in the vagina. Adolescents and women in their pubertal and post pubertal years are characterised by thicker labia, hypertrophied hymens and vaginal walls, a stratified squamous vaginal mucosa, vaginal mucous glands, and a typical vaginal flora dominated by lactobacilli. The main cause of the alterations that result in bacterial vaginosis seems to be the loss of vaginal lactobacilli. Failure to develop a healthy, lactobacilli-dominated vaginal microbiome is linked to recurrent bouts of vaginitis [16].

Vaginal infection

Disorders that result in vaginal inflammation or infection are referred to as vaginitis. Inflammation of the vagina and vulva, the outer region of the labial and skin around the vagina, is referred to as vulvovaginitis. Infections brought on by bacteria, yeast, or viruses, as well as irritations from chemicals in creams, sprays, or even clothing that comes into touch with this area, can cause these disorders. Occasionally, germs that are transferred between sexual partners cause vaginitis [17-18].

Description of vaginitis

When most women hear the phrase vaginitis, they typically think of the term "yeast infection." But there are other types of vaginal infections besides yeast infections. Hormonal fluctuations, allergies, irritations, and several pathogens can all cause vaginitis, sometimes simultaneously. Additionally, the use of antibiotics, diabetes, excessive drinking, steroids, a compromised immune system, vaginal abrasions, or tight non-cotton pants can all contribute to vaginitis.

Causes and risk factors of vaginitis

The six most prevalent forms of vaginitis are as follows. These include: bacterial vaginitis, trichomoniasis vaginitis, chlamydia vaginitis, viral vaginitis, candida or yeast infections, and non-infectious vaginitis.

It is not always easy for a patient to identify which type of vaginitis she truly has, even though the symptoms of each of these vaginal infection types can differ; even a qualified physician may have trouble diagnosing it. Part of the problem is that several types of vaginitis can sometimes coexist. Often, vaginitis presents with no symptoms at all [18].

Vaginitis is frequently caused by yeast infections. The vaginal discharge from yeast infections is thick,

white, and has the consistency of cottage cheese. The discharge is odourless even if it may be a little runny.

The vagina and vulva are typically quite itchy and red when there is a yeast infection. The "friendly" bacteria that typically keep the yeast in balance can be killed by an antibiotic used to treat a urinary tract infection; consequently, the yeast overgrows and causes the illness.

A vaginal discharge is a symptom of bacterial vaginitis. Usually thin and milky, the discharge is occasionally characterised as having a "fishy" smell. This smell could be more obvious after sexual activity. Since bacteria are the cause of bacterial vaginitis, antibiotics are typically used as a treatment.

The single-celled organism that causes trichomoniasis, sometimes known as "trick," belongs to the protozoa family of microbes. A frothy, greenish-yellow discharge may result from this organism's infection of the vagina. This discharge frequently smells bad. Women who have trichomonas vaginitis may experience burning when urinating, as well as itching and pain in the vulva and vagina. Following a menstrual cycle, these symptoms could get worse. Sexual contact can spread this particular form of vaginitis.

Vaginitis can be brought on by the sexually transmitted disease chlamydia. Regretfully, the majority of women show no symptoms. Occasionally, but not usually, this illness is accompanied by a vaginal discharge. Light bleeding is more common in women, especially after sexual activity. The majority of young women under 30 who have several sexual partners are at risk for chlamydial vaginitis [19-20].

Sexual contact can transfer the herpes simplex virus, which can cause viral vaginitis. Pain related to lesions or sores is the main sign of herpes vaginitis. Only a gynaecologic examination can reveal these sores, which are typically found on the vulva or vagina.

Most frequently, an allergic reaction or irritation from vaginal sprays, douches, or spermicidal products results in non-infectious vaginitis. Additionally, the skin surrounding the vagina may be sensitive to fabric softeners, detergents, and soaps with fragrances. There isn't any infection [18].

Symptoms of vaginitis

Itching, burning, and vaginal discharge that differs from the usual secretions are common signs of vaginitis. The burning and itching may occur on the skin or vulva immediately outside the vagina or inside the vagina. Additionally, discomfort may arise

during sexual activity or urinating. The diagnosis would be quite easy if all vaginitis patients had the same symptoms. It's crucial to understand that up to 40% of women who have vaginitis might not exhibit these common symptoms. Even in the absence of symptoms, vaginitis is often confirmed by a routine gynaecologic examination. It is crucial to get a gynaecologic examination on a frequent basis because of this [18].

Treatment of vaginitis

A correct diagnosis is essential for effective treatment. Antibiotics, antifungal creams and suppositories, and other prescription medications may be used because the causes are diverse. Instead of starting with oral medication, vaginal creams and lotions are frequently advised first. *Diflucan*, *azithromycin*, *doxycycline*, *flagyl* (metronidazole), and over-the-counter yeast medicines are among the frequently utilised drugs. Consuming yoghurt that contains live acidophilus cultures and doing vinegar douches or sitz baths in a solution of one teaspoon of vinegar for every gallon of water are examples of self-care practices. The effectiveness of these has not been established by studies.

Sometimes, oral fluconazole (Diflucan), itraconazole (Sporanox), and ketoconazole (Nizoral) or vaginal clotrimazole (Mycelex, Gyne-Lotrimin, Femcare) are used to treat recurrent yeast infections (more than four episodes annually) for six months. It is advised to refrain from having sex until after treatment is finished.

RESULTS AND DISCUSSION

Prevention of vaginitis Overview

Candida albicans is the most common cause of vulvovaginal candidiasis (VVC), while other *Candida* species or yeasts can also cause it. Pruritis, vaginal discharge and occasionally vaginal discomfort, vulvar burning, dyspareunia, and external dysuria are all signs of vulvovaginal candidiasis. Vulvovaginal candidiasis may develop after receiving antibiotic treatment or concurrently with an STD. According to estimates, 40% to 45% of women will experience two or more episodes of vulvovaginal candidiasis, and 75% of women will experience at least one episode. Depending on the host characteristics, microbiology, clinical presentation, and response to treatment, vulvovaginal candidiasis can be categorised as either difficult or uncomplicated. Complicated vulvovaginal candidiasis affects 10% to 20% of women, indicating the need for diagnostic and treatment measures [21,22].

Candida species are members of the *Cryptococcaceae* family. *Candida albicans*, which can produce both

spores and pseudo hyphae, is the species of *Candida* that most commonly infects humans. A common resident of the mouth, throat, large intestine, and vagina is *Candida albicans*. All human mucosal surfaces are colonised by *Candida* species during or shortly after birth, and endogenous infection is always a possibility [22].

Sometimes, a systemic condition (like diabetes mellitus), pregnancy, non-diabetic glycosuria, a diet high in fruit or sugar, debilitation, corticosteroids, broad-spectrum antibiotics, and maybe oral contraceptives are linked to clinical infection [21].

Diagnosis

The diagnosis is made based on both the presence of *Candida* mycelia and the disease's clinical characteristics. The discovery of filamentous forms (pseudo hyphae) of the organism is necessary for the identification of *Candida albicans*. Exudates from the vaginal wall are combined with a 10–20% potassium hydroxide solution, put on a cover slip, and then inspected under a microscope. To improve diagnosis accuracy, a quick microscopic analysis of vaginal discharge combined with 10% Potassium Hydroxide (KOH preparation) and Gramme staining of the discharge are employed [20,22].

Morphology and identification

Candida species develop as oval budding yeast cells (3-6 micrometres in size) in culture or tissue. Additionally, they produce pseudo hyphae, which are chains of elongated cells that are pinched or constricted at the cell-to-cell junction, when the buds expand but do not separate. *Candida albicans* is a dimorphic organism that may produce real hyphae in addition to yeasts and pseudo hyphae. Within 24 hours at 37° C or room temperature, or on agar media, *Candida* species form soft, cream-coloured colonies that smell like yeast. Below the agar surface, pseudo hyphae appear as submerged growth [20,22].

Classification of Vulvovaginal Candidiasis (VVC)

Simple VVC; sporadic or occasional vulvovaginal candidiasis; mild-to- moderate candidiasis; women who are not immunocompromised; and a higher likelihood of *Candida albicans*

Infertility, recurrent vulvovaginal candidiasis, severe vulvovaginal candidiasis, non-*albicans* candidiasis, women with uncontrolled diabetes, immunosuppression, or those who are pregnant

Treatment of vulvovaginal candidiasis

A number of topical medications are still advised as first-line treatments for pregnant women with

vulvovaginal candidiasis. Fluconazole (Diflucan), an oral medication, has just been approved for the treatment of vulvovaginal candidiasis [20,23]. When using oral medicines, systemic effects, side effects, and drug interactions need to be taken into account. For simple vulvovaginal candidiasis, short-course topical formulations (i.e., one dosage and regimens of one to two days) work well. Nystatin is less effective than the azole medications used topically. 80% to 90% of patients who finish treatment with azoles report a reduction in symptoms and negative cultures. There are Over-The-Counter (OTC) preparations for the intravaginal administration of *butaconazole*, *clotrimazole*, *miconazole*, and *tioconazole*. Only women who have previously been diagnosed with VVC and experience a repeat of the same symptoms should be encouraged to self-medicate with over-the-counter medicines. Any woman who experiences a recurrence of symptoms within two months or whose symptoms do not go away after taking an over-the-counter treatment should consult a doctor. OTC preparations are frequently used inappropriately or needlessly, which might cause treatment delays for various vulvovaginitis aetiologies and have a negative therapeutic impact.

Special considerations intolerance or allergy to the suggested treatment. Despite the possibility of local stinging or irritation, topical agents often have no systemic side effects. Sometimes, oral medications result in headaches, nausea, and stomach pain. Rarely, aberrant increases of liver enzymes have been linked to oral azole therapy. Combining these oral medications with other medications such as astemizole, calcium channel antagonists, cisapride, coumadin, cyclosporin A, oral hypoglycaemics agents, phenytoin, protease inhibitors tacrolimus, terfenadine, theophylline, trimetrexate, and rifampin may result in clinically significant interactions [20,22].

Torch test

The TORCH test falls within a group of blood tests that identify the concentration of an infectious disease antibody titer in blood or serum. The initial letters of the five chronic infections—Herpes Simplex Virus (HSV), Rubella, Cytomegalovirus (CMV), and Toxoplasmosis—are combined to form the acronym TORCH 33 Syphilis (TORCHS), Parvovirus B19, Enterovirus, Coxsackie virus, Epstein-Barr virus (mononucleosis), Hepatitis B, and Human Immuno Virus (HIV) have since been included as "others" to the acronym (CHEAP TORCHS) [24,25].

TORCH Syndrome is a collection of symptomatic birth abnormalities that can be brought on by multiple TORCH infections 35. For neonates with

congenital malformations and females with Bad Obstetric History (BOH), the TORCH test is typically recommended. The majority of practitioners, however, do not fully comprehend the test, which leads to the widespread practice of indiscriminately requesting the tests, which results in resource losses. Because they don't fully comprehend the TORCH test, practitioners from the ObG, Paeds typically recommend it for newborns with congenital malformations or for females with BOH (Bad Obstetric History). With the current knowledge of TORCH agents and their clinical manifestations, it is now required to assess the TORCH test's usefulness in order to eliminate the possibility of misunderstandings and conserve test resources. The TORCH test has become the most misused test because of this [26,27]. The usefulness of this testing in clinical settings has been questioned in many places, as is well known Microbiologists claim that because these demands are not properly targeted, they are frequently unsuitable [28,29].

To detect infections in expectant moms, a set of tests known as a TORCH screen is utilised. Infections can be passed on to the growing baby during pregnancy. Early infection detection and treatment can prevent newborn issues.

The illnesses covered by the screening are shortened to TORCH, which is often spelt TORCHS: Additional toxoplasmosis types, such as HIV, varicella, hepatitis viruses, and

parvovirus German measles (rubella) The cytomegalovirus Herpes simplex Syphilis

Diseases detected by TORCH screen Toxoplasma Toxoplasmosis can result from a parasite (*T. gondii*) entering the body through the mouth. The parasite can be found in cat litter and excrement, raw eggs, and undercooked meat. Infants who contract toxoplasmosis during pregnancy usually show no symptoms for several years. During pregnancy or childbirth, the mother may transmit any of these disorders to the baby.

MATERIALS AND METHODS

Method of preparation of herbal formulations/ preparation of plant extract

Betel leaves extract:

Fresh Betel Leaves



Washing Leaves



Arranging Leaves

b) A filter paper bag containing the finely ground powder was placed into a Soxhlet device and filled with 100 millilitres of ethanol. The vapours condensed after the extraction solvent in the flask was heated.

c) When the liquid level reached the top of the syphon tube, the contents of the chamber syphon were gathered into a flask. Until the syphon tube was entirely empty, this process continued. Crude extract was obtained and put in a round-bottom flask once the collected extract had completely evaporated all of the solvents using Rotavapoevaporation [30].

Tea Tree Oil

Oil extraction via Soxhlet extraction a sample of 50 grams of refined tea tree powder was weighed, put into a thick cellulose thimble, and then put into the Soxhlet extractor chamber. After that, the extractor was heated to its boiling point temperature and attached to a 500 ml flask that had 300 mL of n-hexane solvent. Six extraction cycles, or roughly six hours, of the extraction procedure were carried out [31]. The collected mixture was further purified using a rotary evaporator under vacuum conditions for the solvent removal procedure at 40°C after the extraction process. This technique ultimately produced a light-yellow waxy texture that is known as tea tree concrete.

To remove the natural waxes and other heavy albuminous chemicals, the concrete residue was further dissolved in 6 mL of methanol solvent. The wax molecules were then precipitated by heating the mixture to 40°C for 30 minutes and then cooling it to - 15°C for 24 hours [32]. The blend of essential oils and methanol solvent is then extracted from the waxes and other resin components by vacuum filtration. The generated essential oils, along with a few wax traces, can be referred to as tea tree absolutes after the filtrated combination was once more refined using rotary evaporation to eliminate the excess methanol solvent [33]. The components of interest (terpinen-4-ol, 1, 8-cineole, γ -terpinene, and α -terpineol) were identified by further analysing the acquired tea tree absolutes under GC-MS component characterization.

Preparation of Shatadhauta Ghrita

Hundred washes of ghee" (Shatadhauta Ghrita) is an Ayurvedic preparation that is created by washing cow's ghee with water 100 times:

Make sure you choose premium ghee: Select premium cow ghee for the procedure.



Wash in a copper vessel: Traditionally, a copper vessel is used for the washing procedure. .



Melt and combine: Combine water and melted ghee.



Eliminate contaminants: Remove contaminants from the ghee.



Repeat: Repeat the process 100 times.



Sun-dry: To get rid of any last bits of moisture, sun-dry the ghee after washing.



Store: Keep the dried ghee in a hygienic, tightly sealed container.

It is said that the washing process will make the ghee more stable; make it suited for topical treatment; speed up healing; and lessen scarring and burning.

Herpes, leprosy, burns, wounds, scars, and chicken pox can all be treated with shata dhauta ghrita. Some Ayurvedic healthcare companies also sell it as a moisturizer.

Preparation of solid nutrient media using agar as nutrient:

All ingredients except agar dissolved in specified amount of distilled water.



Adjust the p^H and add agar powder.



Treat the medium to dissolve to form clear liquid.



Autoclave

Results after staining of petri plate after incubating for 24 hours (Supplementary Tables 1-7) (Supplementary Figures 1-14).

CONCLUSION

The present study demonstrates that the polyherbal cream containing Ajwain (*Trachyspermum ammi*), Betel Leaf (*Piper betle*), Neem Oil (*Azadirachta indica*), and Tea Tree Oil (*Melaleuca alternifolia*) is a safe and effective formulation for managing vaginal irritation and yeast infections. Women suffering from these conditions often experience discomfort, itching, and soreness, which significantly impact their quality of life. Conventional treatments, while effective, are sometimes associated with side effects, recurrence, and antibiotic resistance, highlighting the need for alternative therapeutic options. The polyherbal cream offers a natural solution that alleviates symptoms while minimizing potential adverse effects. Its combination of medicinal plant extracts provides antimicrobial, anti-inflammatory, and soothing properties, contributing to symptom relief and improved vaginal health. Overall, this study supports the potential of polyherbal formulations as complementary or alternative therapies in gynecological care. Further clinical studies are recommended to validate its efficacy and safety for widespread use.

DECLARATIONS

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Author contributions

All authors contributed equally to the preparation of this manuscript and approved the final version for publication.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

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Data availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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