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Herbal Vaginal Cream

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Abstract:

Vaginal infections are very common in females of almost all the ages. It may be caused by bacteria, yeast and other micro-organism. There are various allopathic medicine such as anti-fungal, anti-bacterial drugs available to treat the same, but all these drugs give short time effect to treat various diseases. Also, Herbal phytochemicals are more effective as compared to synthetic drugs. The present paper was designed to enumerate few herbs and their use for the treatment of vaginal infections. In the present communications method of preparation of Aloe barbadensis miller, Amla, Withania Somnifera (Ashwagandha) was discussed to be used in vaginal infection.

Keywords: Vaginal infections, Treatment, Herbs, Candida Vaginitis, Rosemary.

Introduction

Vaginal infections are caused by microorganisms; infections. Usually cause a discharge with itching, redness, and sometimes burning and soreness. However, these symptoms do not necessarily indicate an infection. Instead, they may result from other conditions that affect the vagina. For example, chemicals or other materials (such as hygiene products, bubble bath, laundry detergents, contraceptive foams and jellies, and synthetic wear) can irritate the vagina and cause a discharge and discomfort. Inflammation thus results are known as noninfectious (inflammatory) vaginitis. A vaginal discharge may be caused by a disorder that affects other reproductive organs, rather than the vagina. For example, a discharge can result from certain sexually transmitted diseases such as chlamydial infection or gonorrhea. The bacteria that cause these diseases can spread from the vagina to the cervix (the lower, narrow part of the uterus that opens into the vagina) and the uterus, causing PID genital herpes, which can cause blisters on the vulva (the area around the opening of the vagina), in the vagina, and on the cervix, can also cause a vaginal discharge. Causes of vaginal infection. The main causes of vaginal infections are:

- Bacteria
- Yeast
- Other microorganisms.

Vaginal diseases:

- Vulvovaginal candidiasis
 - Bacterial vaginosis
 - Chlamydia
-
- ❖ Aloe vera – *Aloe barbadense miller* – Liliaceae family
 - ❖ Ashwagandha – *Withania Somnifera* – Solanaceae family
 - ❖ Amla – *Phyllanthus embolic L.* – Euphorbiaceous family

Preparation of Aloe Vera Leaf Extract:

Plant collection: The aloe vera leaves were collected from Sinhgad institute of Pharmacy, Narhe Medicinal Garden, Pune, India.

Preparation of plant extract: The aloe vera leaf was cut at the base of the plant, lower leaf was sliced and juice was collected.

Extraction of Aloe Vera:

Mature, healthy and fresh aloe vera leaves was collected and washed with distilled water. Then after proper drying of leaves in hot air oven, the outer part of the leaf was dissected longitudinally using a sterile knife. Then the aloe vera gel that is the colorless parenchymatous tissue was removed using the sterile knife. Then it is filtered using muslin cloth to remove the fibers and impurities. Then the filtrate or the filter product which is a clear aloe vera gel was used in the preparation.



Fig.1 Aloe-vera extraction



Preparation of herbal vaginal cream:

Cream: Cream is defined as semi- solid emulsion which could be oil in water (o/w) or water in oil (w/o) type.

Procedure of herbal vaginal cream:

Heat liquid paraffin and beeswax in a borosilicate glass beaker at 75 °C and maintain that heating temperature. (Oil phase). In another beaker, dissolve borax, methyl paraben in distilled water and heat this beaker to 75 °C to dissolve borax and methyl paraben and to get a clear solution. (Aqueous phase). Then slowly add this aqueous phase to heated oily phase. Then add a measured amount of aloe Vera gel, Ashwagandha, amla, Rosemary oil and stir vigorously until it forms a smooth cream. Then add few drops of Lavender oil as a fragrance and carbol fusion as colourant.

Ingredients table:

Table No. 1 Ingredient table

Ingredients	Quantity	Role
Aloe vera	3 ml	Anti-inflammatory
Amla	2 ml	Antifungal, Antimicrobial
Ashwagandha	3.8 ml	Antifungal, Antimicrobial
Methyl Paraben	0.02 ml	Preservative
Borax	0.2 ml	Emulsifying agent
Liquid paraffin	8 ml	Lubricating agent
Bees Wax	2 ml	Emulsifying agent
Lavender oil	q. s	Anti-fungal activity
Rosemary oil	q. s	Anti-fungal activity
Safranin	q. s	colourant

Antimicrobial Activity of Cream Formulations:

The antibacterial activity of cream formulations was performed by standard agar well diffusion method against staphylococcus aureus, E. coli and bacillus. Nutrient broth/agar was used to cultivate bacteria. In order to recover the lyophilized culture, the desire amount of culture was aseptically transferred in nutrient broth and maintained in an incubator at 37° C for 3 hrs. to form inoculums. The

media was poured in petri plates aseptically and kept for 30 minutes for solidification. After 30 minutes, the fresh inoculums of different culture were spread on to solidified nutrient agar plates. Wells were made using sterile small test tubes of 5mm diameter on petri plates at proper positions. The cream was added in to agar wells, aseptically. Then the agar plates were incubated at 37°C for 24hrs. After 24 hrs. of incubation, the zone of inhibition was investigated.

Nutrient medium:

Beef extract- 1gm

Peptone- 1gm

NaCl- 0.5gm

Distilled water - 100ml

Agar- 3gm



Fig.2 *Bacillus lactobacillus*



Fig.3 *S. Aureus*

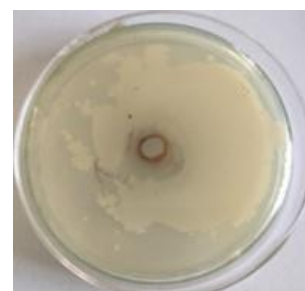


Fig.4 *E. coli*

Result:

Table No. 2 Antimicrobial activity

Micro-organism	Zone of inhibition
E. coli	18mm
S. Aureus	17mm
Bacillus	22mm

Anti-Fungal Activity of Cream Formulations:

The anti-fungal activity of cream formulations was performed by standard agar well diffusion method against *C. albican*. Nutrient broth was used to cultivate fungi. In order to recover the lyophilized culture, the desire amount of culture was aseptically transferred in nutrient broth and maintained in an incubator at 37° C for 3days to form inoculums. The media was poured in petri plates aseptically and

kept for 30 minutes for solidification. After 30 minutes, the fresh inoculums of culture were spread on to solidified sabouraud dextrose agar plates. Wells were made using sterile small test tubes of 5mm diameter on petri plates at proper positions. The cream was added in to agar wells, aseptically. Then the agar plates were incubated at 37⁰C for 48hrs. After 48 hrs. of incubation, the zone of inhibition was investigated.

Nutrient medium:

Sabouraud dextrose Agar - 6gm

Agar- 3gm



Fig.5 Candida albicans

Result: Zone of inhibition of candida albicans was found to be 19mm.

Comparison of antifungal activity of herbal vaginal cream and marketed cream:

Zone of inhibition of herbal vaginal cream was found to be 20mm and marketed was found to be 16mm. Hence, we conclude that herbal vaginal cream antifungal activity is more compared to marketed cream.



Fig.6 Antifungal activity of herbal vaginal cream and marketed cream

Phytochemical Tests:

A) Phytochemical Test for Amla:

1)Test for Tannin:

To 1 ml of extract, 2 ml of 5% FeCl₃ was added. A dark blue or green black colour indicates the presence of tannins.



2) Test for Carbohydrates:

- a) Molisch's test: -To the extract add few drops of alcoholic α -naphthol, then add few drops of concentrated sulphuric acid through sides of test tube, purple to violet colour ring appears at the junction.
- b) Fehling's test: - Mix 1 ml Fehling's A and 1 ml Fehling's B solutions, boil for 1 min. add equal volume of test solution. Heat in boiling water bath for 5-10 min. first a yellow, then brick red precipitate is observed.
- c) Benedict's test: -Mix equal volume of Benedict's reagents and test solution in test tube. Heat in boiling water bath for 5 min. solution appears green, yellow or red depending upon amount of reducing sugars present in test solution.

3) Test for Flavonoids:

Shinoda test: - To the extract add few magnesium turnings and concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

4) Test for Steroids:

Lieberman-Burchard test: - Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids.

B) Phytochemical Test for Ashwagandha:

1) Test for Phenol:

Ferric Chloride test- Take 2 ml of Ashwagandha filtrate in a test tube and then add 2 ml of ferric chloride (1%). The appearance of dark green or bluish green colour indicated the presence of phenol.

2) Test for tannins:

Add few drops of lead acetate solution in a test tube with 2 ml of filtrate. Yellowish coloration was indication of positive result.

3. Test for Flavonoids:

Alkaline Reagent Test - Take the 200 mg of extract in a test tube add few drops of Sodium hydroxide solution. Then add few drops of dilute hydrochloric acid, change in the colour from deep yellow colour to colourless indicates the presence of flavonoids.

C) Aloe vera test:

1) *Borax test*: Heat 5ml solution with 0.2 g borax, add few drops of this test tube filled with water- green fluorescence is produced.

2) 5ml solution + 2ml nitric acid -yellow-brown color produced.

3) *Nitrous acid test*: Solution + NaNO₂ + acetic acid heat - reddish-brown color.

4) Aqueous aloe vera solution+ 5% copper acetate + saturated NaCl solution + alcohol, warm- deep wine-red color.

Test results for Ashwagandha:

Table No. 3 Phytochemical test for Ashwagandha

Test	Result
Ferric chloride test	Positive
Alkaline reagent test	Positive
Tannin test	Positive

Test results for Amla:

Table No. 4 Phytochemical test for Amla

Test	Result
Tannins test	Positive
Molish's test	Positive
Fehling's test	Positive
Fehling's test	Positive
Shinoda test	Positive
Liebermann-Burchard test:	Negative

Evaluation of Cream:

1) **Organoleptic properties**: In this test, the cream was observed for color, odor, texture, state.

2) **Wash ability**: A small amount of cream was applied on the hand and it is then washed with tap water.

3) **pH**: 0.5 g cream was taken and dispersed in 50 ml distilled water and then pH was measured by using digital pH meter.



Fig.7 pH meter

4) Spreadability: Adequate amount of sample is taken between two glass slides and a weight of 100gm is applied on the slides for 5 minutes. Spreadability can be expressed as,

$$S = M \cdot l / t$$

Where, m = weight applied to upper slide. l = length moved on the glass slide.

t = time taken.

5) Homogeneity: The formulation was tested for the homogeneity by visual appearance and by touch.

6) Removal test: The ease of removal of the creams applied was examined by washing the applied part with tap water.

7) Dye test: The scarlet dye is mixed with the cream. Place a drop of cream in a slide and cover with a cover slip and examine it under a microscope. The disperse globule appears red and the ground colourless then it is o/w type cream.

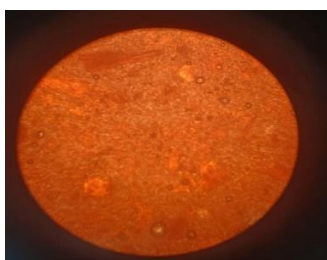


Fig.8 Dye test

8) Saponification value: 2gm of substance refluxed with 25ml of 0.5 N alcoholic KOH for 30min, to this 1ml of phenolphthalein added and titrated immediately, with 0.5N HCl, note the reading as 'a'. Repeat the operation omitting the substance being examined. Note the reading as 'b'.

$$\text{Saponification value} = (b - a) \cdot 28.05 / w$$

Where, W = weight of substance in gram.



Fig.9 Saponification test

9) Phase separation: The cream is kept in a closed container at 25-30⁰C not exposed to light. Phase separation was observed regularly for 30 days any kind of phase separation is observed.

10) Stability- The study was carried out for 2 months and no change was noticed. The stability studies were carried out by storing at different temperature conditions like 20⁰C,25⁰C,40⁰C for 2 months.

Summary of evaluation parameter:

Table no.5 Evaluation parameter test

Parameters	Observation
Color	Light Pink
Odor	Lavender odor
Texture	Smooth
PH	4.18
Spreadability	1.2
Homogeneity	Homogenous in nature.
Removal test	Easy to remove
Dye Test	o/w type
Saponification Value	28
Phase Separation	No phase separation observed
Stability	30 days observed



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Conclusion: This study revealed the presence of important Phytochemical of aloe vera. Amla and ashwagandha were effective to treat various vaginal infection. The herbal vaginal cream is more effective to treat bacterial as well as fungal infection than synthetic cream. various evaluation parameters such as physical properties, PH, spreadability, washability, non-irritancy test, viscosity and phase separation of cream and gives excellent results. This herbs play very crucial role from medicinal point of view and could be used as alternative to synthetic cream that will help to reduce their toxicity in medications. It also highly recommend to treat infection of candidiasis albicans.

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