Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 4920 - 4929
Received 15 December 2020; Accepted 05 January 2021.

Formulation and Evaluation of Feminine Wash for Candidiasis with the Extraction of Piper Betle as Antifungal Effect

Faiza Naimat[1], Nur Annissa[2],Aqielahbinti Muhammad Zaki[3],P.M.Ridzuan[4]
[1,2,3]School of Pharmacy, Management and Science University, Seksyen 13,40100 Shah Alam, Selangor, Malaysia
[4] International Medical School, Management and Science University, Seksyen 13, 40100 Shah Alam, Selangor, Malaysia

ABSTRACT

Background and Aims : Piper betle leaves is the medicinal plant that has been used as the traditional medicines as an antifungal to help vagina or oral candidiasis.The extract of the Piper Betle has significant functions that may help as an anticandidal effect against the Candida albicans, and Candida glabrata, The study was designed to formulate and evaluate the characterization and the effectiveness of the feminine wash with the extraction of plants Piper Betle.L that may help in treating the Candidiasis that provides the best patient compliance with the minimal side effects. Method: The maceration method was used to prepare the extract. Three different concentrations of Piper Betle feminine wash, such as 250 mg/ml, 125 mg/ml and 62. 5 mg/ml were formulated and tested with the Candida Albicans sp..using disc diffusion method and zone of inhibition was compared using negative control. The physical evaluation of preparation of feminine wash including the pH test, appearance, and color were conducted. Result and Discussion: The formulation of 62.5 mg/ml feminine wash with Piper betle extract was effective against the Candida Albicans sp. with the diameter zone inhibition of 19 – 21 mm. This indicates that the formulation with 62.5 mg/ml of extraction is effective and can be used as topical drug delivery. Conclusion: It is found that the concentration that is most effective against the antifungal properties was 62.5 mg/ml Piper Betle Feminine wash formulation.

Keywords
Piper Betle, Antifungal, Feminine wash, Candidiasis

I. Introduction

Women typically use feminine hygiene products as part of their daily routine. Many factors affected these behaviors, including personal interest, cultural values, religious beliefs and advice from health care professionals. The vaginal environment is prevalent in literature, little is known about the vulvar area and how personal hygiene practices can influence its biological and physiological stability. Specifically, there will be more about intimate feminine hygiene as it relates to external topical washes and the role of intimate feminine hygiene in managing unpleasant symptoms and promoting overall intimate health. (Chen, Bruning, Rubino, & Eder, 2017)

Vagina is an organ that is important to women as a source of pleasure, but also a source of potential pain. It is open to the outside and located near the anus, where the number of potentially pathogenic bacteria is large. Therefore, a vaginal protection system is needed to prevent the

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 4920 - 4929
Received 15 December 2020; Accepted 05 January 2021.

growth of pathogens in the body. Although the protection system is already in place, vaginal discomfort is the most common cause of gynecologist visits. Vaginal protection systems are based on the maintenance of 3.5 to 4.5 acidic pH, whereas the common and potentially pathogenic saprophytes do not detect proliferating conditions. (Haya, García, López-manzanar, Balawi, &Haya, 2014)

The vulva is the first line of defense that protects the genital tract from the infection. The contaminants are often collected in the vulvar folds and increased the moisture, sweating, menses and hormonal fluctuations that may be influenced by the vulvar microbial growth and species balance, potentially may be resulting in odor and vulvovaginal infection. Vulvar skin differs slightly from other skin sites in hydration, friction, permeability and visually irritation and is more susceptible to topical agents than forearm skin because of its increased hydration, occlusion and frictional properties. The non-keratinized vulvar is likely to be more permeable than keratinized skin. Genital skin is unique in that it is covered by a thin stratum corneum that contains large hair follicles, which will make it easier for microbes to permeate the skin. The vagina is the fibro- muscular canal that extend from its external opening in the vulva to the cervix and is composed to mainly of smooth muscle that are covered with a non-keratinized epithelial lining, at which until menopause, is thick with fold kept moist by the fluid secreted through the vaginal wall and mucus from the cervical and vestibular glands. (Chen et al., 2017)

Vulvovaginal candidiasis is a type of fungal infection of the vagina or perineal area that accounts for approximately one-third of cases of vaginitis. The most frequent cause of candida vulvovaginitis is candida albicans. The usual clinical is that of itching, burning, and erythema. It is not considered as a sexually transmitted disease. (Jeanmonod.R, &Jeanmonod.D, 2019).Vulvovaginitis, or inflammation of the vulva and vagina is the most commonly secondary to infectious agents in reproductive aged women.(Rebecca Jeanmonod; Donald Jeanmonod., 2019).*Candida* is a type of fungi that is well-known which consists of around 20 pathogenic species. That area belongs in a class of *Saccharomycetes*with a family of *Saccharomycetaceae*.(Shao, Sheng, & Zhang, 2007). It is stated as the *Candida* because of the ailments that are caused from the mucocutaneous tissue and the bloodstream infections. (Eggimann, Garbino, &Pittet, 2003)

Fungal disease of vulva and vagina is related to the species Candida (vulvovaginal candidiasis) which is the most common female mucosa. IIt shows the acute or chronic courses and patterns of disease that can have a strong impact on the quality of life of the women concerned. In general, the most common cause of acute vulvovaginal candidiasis is *Candida Albican*, followed by *Candida glabrata*. Albicans and C.glabrata are often equally distributed. (Stock, 2010).In the last few decades, fungal infections have increasingly been recognized as a major health threat to an ever-expanding list of compromised patients.(Brown et al., 2012). *C.albicans*is a part of normal human microbiota that is usually acquired early in neonatal life and as a normal commensal that colonizes mucosal surfaces, that is particularly those of skin and produces a little to no damage to the host.(Brown et al., 2012).

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 4920 - 4929
Received 15 December 2020; Accepted 05 January 2021.

II. MATERIALS AND METHODS

Plant Materials

Leaves of *Piper Betle*collected from Kelantan and has been verified by Universiti Putra Malaysia (UPM) with MFI Voucher No 0026/18.

Chemical Materials

The chemicals such as sodium lauryl sulfate (6.0 % active), cocamidopropylbetaine (5.0% active), germall plus, sodium chloride, citric acid, plant extract and distilled water were used in the formulations.

Equipment

Blender, Refrigerator, Freeze Dryer

Preparation of *Piper Betle L.*

The crude extract was prepared based on the procedures that are conducted by the supervisor. In subsequent time, the leaves and branch was been separated and was been dried overnight by using an oven with the constant temperature that has been set, 40^{o}C. After the leaves were dried, the leaves were grinded until the fine powder was produced.

Preparation of the crude aqueous extract of *Piper Betle L.* by maceration and freeze drying method

Apart from that, the crude fine powder that has been grind was extracted with distilled water for two days. After the extraction period, the solution was filtered and transferred. To obtain pure, powdered extracts, the filtrate was collected and evaporated by using a freeze dryer. For further usage all the extracts were kept at 4°C.

Preparation of the formulation of feminine wash

The first steps for feminine wash preparation started with the addition of Sodium Lauryl Sulfate and CocamidopropylBetaine and were dissolved in the water and stirred homogeneously. In the above mixture the preservatives such as germall plus and Piper Betle extract were dissolved and stirred homogeneously. Next steps, sodium chloride was added as the functions of controlling the formulations 'viscosity in the mixture above. Last steps for feminine wash preparations were added citric acid to the mixture above. Citric acid is necessary because it controls the pH of the formulations using its purpose.

Ingredients	**F1 Conc. % w/w**	**F2 Conc. % w/w**	**F3 Conc. % w/w**
Sodium Lauryl Sulfate	5.5	5.5	5.5
Cocamidopropyl sulfate	5.5	5.5	5.5

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 4920 - 4929
Received 15 December 2020; Accepted 05 January 2021.

Germall plus	0.05	0.05	0.05
Citric Acid	qs	qs	qs
Sodium chloride	2.0	5.0	5.0
Gel thickener	-	10	10
Water	qs to 100 mL	qs to 100 mL	qs to 100 mL

Table 1**: Composition of the feminine wash formulations**

Bacteria culture and Growth

A bacterial culture is a method used by bacterial organisms by enabling them to reproduce under controlled laboratory conditions in predetermined culture media. It is important to provide the correct environmental and nutritional conditions which exist in its natural habitat for any bacterial culture.

1. Firstly, use a disinfectant solution to clean all work surfaces.
2. Flame an inoculating loop at the bunsen burner. Remove the cap or cotton plug with the "loop" hand and flame the mouth of the tube.
3. Put the cooled loop into the tube of the stock that contains fungi, *C. albicans*and collect a small amount of the crop (more than for transfer to a tube).
4. Touch the loop to the top of the platter and streak from side to side all the way down to the bottom. The finished plate has a zigzag pattern from border to edge.

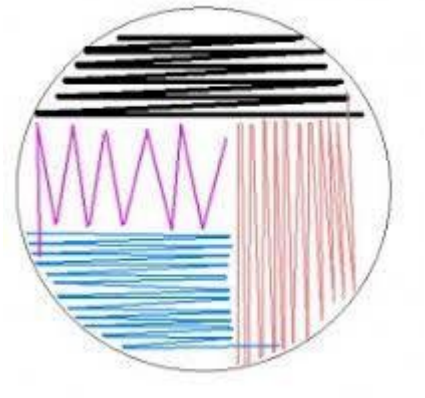

Fig. 1**: Sample of the isolation streaking techniques.**

5. The cover was lowered and the loop was flamed. Then all petri dishes incubated at temperatures above 40°C should be set upside down.
6. Next steps, hold the tube of the stock into one hand. Flame the loop again, and removed from the tube the cap or plug. The tube's mouth flames. Insert the cooled loop into the tube and remove a small amount of the same as for a tube.
7. In a zigzag pattern, streak only the top one-fourth of the plate and replace the cover.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 4920 - 4929
Received 15 December 2020; Accepted 05 January 2021.

Remove the loop, flame it and let it cool off. Turn the platter 90 ° on the other side of the plates.

Analysis of Minimum Inhibitory Concentration (MIC) of the extraction

MIC testing was performed using a method of broth microdilution. Started the preparation of the serial dilution of the extraction of *P. Betle*that contains five different concentrations (500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL and 31.25 µg/mL). Therefore, the first steps for the MIC test is 150 µL of the SA broth was used as the medium for the cultivation and it was put into the 96-well plates. Next, hold the tube that contains *C. albicans*and swabbed with cotton swab and dipped into the SA broth. After that, take out 20 µL at every concentration and put into the plates that contain SA broth and incubate for 24 hours with the temperature 28 ℃. Lastly, after 24 hours of incubation, 1-2 drops of MTT were dropped into the sample and continued incubating for 15 minutes and results were observed.

Analysis Disc Diffusion Method for Antibacterial Activity

A test of the antibiotic sensitivity of bacteria is the disk diffusion test, or agar diffusion test, or Kirby – Bauer test (disc-diffusion antibiotic susceptibility test, antibiotic sensitivity test, KB test). The preparation of the test was started with dividing the agar plate into 4 parts to see the comparisons between 3 different concentrations and the formulations without concentration. Firstly, using an aseptic technique, placing a sterile cotton swab in the SA broth culture of a *C. albicans*and gently removing the excess liquid by gently pressing or rotating the swab against the inside of the tube. Next, the agar plate was streaked by using the cotton swab to form a bacterial lawn. This rotation repeated for three times. Let the plate dry for 5 minutes and dispense the sterile disc that has been soaked into the different concentration and the blank formulations onto the plate at the different parts that have been divided. And lastly, plates need to be incubated at an incubation temperature of 37°C overnight.

Physical Evaluation of Formulation

Preliminary review was carried out for Formulation 1.

Formulation pH

Digital pH meter was used to determine the pH of formulations. The pH shall be assessed for two weeks.

Formulation of the Physical Texture or Appearance

The formulation was kept at 25°C room temperature and 3°C cool temperature, and regularly checked for 2 weeks. Some changes were detected and noted in the texture of the formulations. The changes on the texture of the formulations reflect the formulations 'physical instability.

Formulation Color

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 4920 - 4929
Received 15 December 2020; Accepted 05 January 2021.

The method for this examination is exactly the same as the observations on texture. The formulation was kept at 25 ° C room temperature and 3 ° C cool temperature, and will be tracked for 2 weeks. Any formulation color changes was observed and noted. The changes on the formulation's color would indicate the formulation's physical instability. Therefore, the changes could indicate the contamination during the formulation.

III. RESULTS

Physical Appearance

FORMULATION	TEXTURE	COLOUR	HOMOGENEITY
F1	Liquid	Clear	Very good
F2	Semi-liquid	White	Good
F3	Gel-like	White	Poor

Table 2**: Results for Organoleptic Evaluation**

pH Result

Formulation	Reading 1	Reading 2	Reading 3	Average
F1	3.8	3.9	4.0	3.8
F2	4.9	4.9	4.0	4.6
F3	4.5	4.7	4.9	4.7

Table 3: **pH Result**

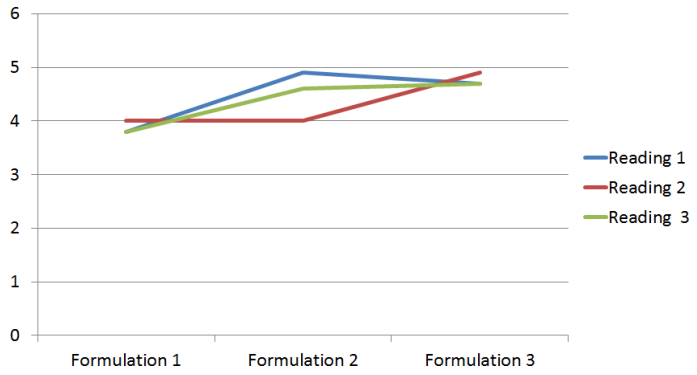

Fig. 2: **Graph for pH result**

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 4920 - 4929
Received 15 December 2020; Accepted 05 January 2021.

Antimicrobial test for the extractions by using broth dilution method

This antimicrobial test for the extraction was done by using broth dilution method. But this test was done by referring to the other research studies of International Medical School students.

Antimicrobial test for the formulations by using disc diffusion

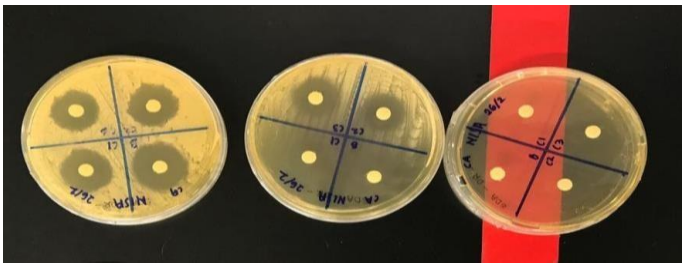

Fig. 3: Disc Diffusion Test

Concentration µg/mL	Reading 1	Reading 2	Reading 3	Average
250	19	19	19	19
125	18	19	19	18.6
62.5	20	21	19	20
Base	20	19	20	19.6

Table 4: Result for antimicrobial test by using disc diffusion

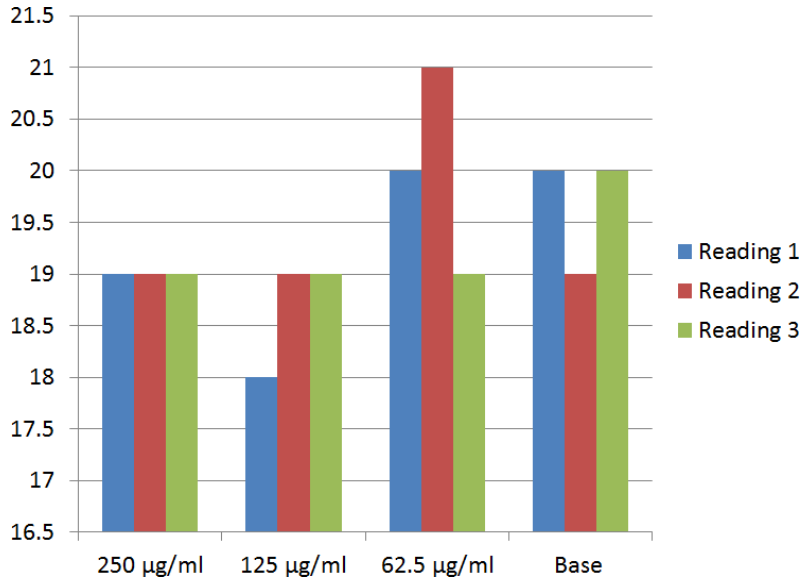

Fig. 4: Graph for antimicrobial test by using disc diffusion method

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 4920 - 4929
Received 15 December 2020; Accepted 05 January 2021.

IV. DISCUSSION

Physical appearance

The physical appearance for the formulations of feminine wash that was examined through the homogeneity of the feminine wash, colour of the formulation, and the texture of the formulation. Firstly, the feminine wash's homogeneity was tested using the foam testing tool to see whether the formulations are well homogeneous. Therefore the results for this homogeneity test were tested as very good, good and poor. Besides that the colour of the formulation was checked by using the appearance test. The formulations of the feminine wash was applied to the skin to examine the texture of the formulation.

Therefore, the result above has shown that Formulation 1 (F1) has a very good homogeneity, it has a clear colour of the base that is suitable for feminine wash. While, the texture of the formulation is in a liquid base.Whereas the Formulation 2 (F2) was less homogenised compared to the F1 which has a slightly white color of the base and the texture that appears in the form of semi-liquid and a bit thicker because of the effect of the reactions between gel thickener and sodium chloride. In the Formulation 3 (F3) that shows the formulation of the feminine wash are less homogenized compared to the F and it has a slightly white colour also same as the F2 and the texture appears in the form of gel-like and also a bit thicker because of the effect from the gel thickener and sodium chloride. Hence, by referring to the result it can be concluded that F1 has a good physical appearance compared to the other formulations.

pH for the formulation

Based on this study, the pH of all three formulations was determined using a standard buffer solution to calibrate the pH meter, and approximately 0.5 ml of each formulation was determined and dissolved in 50.0 mL of distilled water. pH is the number representing the acidity or alkalinity of a solution on a logarithmic scale at which 7 is referred to as neutral, lower values are more acidic and higher values refer to more alkaline. According to Rubino J & Eder, 2017, vulvar pH for feminine wash could be expected to fall between skin values that are estimated at pH of 4.7 and vagina is in the average pH 3.5 with reports ranging from 3.8 to 4.7 during the menstrual cycle. Therefore, by referring to the results, it shows that all three formulations of the feminine wash have shown an average result of pH value of 3.8, 4.6 and, 4.7 respectively. It also can be concluded that all of the formulation has a good and suitable pH to be used for our skin vagina.

The Evaluation of Antimicrobial activity between the concentration studies by MIC

Based on the study, the procedure for this test was evaluated using the method of broth dilution obtained by other students from the International Medical School of Management and the University of Science. The procedure is based on five different concentrations, 500 µg / ml, 250 µg / ml, 125 µg / ml, 62.5 µg / ml and 31.25 µg / ml. The result shows the changes in the color of the MTT reagent affecting all of the above concentrations except for the 62.5 µg / ml concentration, and it can be concluded that the bacteria inhibited at the 62.5 µg / ml concentration.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 4920 - 4929
Received 15 December 2020; Accepted 05 January 2021.

V. CONCLUSION

The research has been conducted to formulate and evaluate the feminine wash for the treatment of *Candidiasis* with the extraction of *Piper Betle*that act as the antifungal effect. Through the evaluation, all of the formulations have shown good remarks in physical appearance such as the homogeneity of the formulations, texture and colour of the formulation. In conclusion, feminine wash containing *Piper Betel* extract has retain the antifungal properties of the extract. The lowest concentration of the extract exhibit a good antimicrobial zone inhibition. It was found that the most effective concentration against the antifungal activity was the formulation feminine wash with the
62.5 µg/mL extract of Piper *Betel.*

VI. REFERENCE

[1] Brown, G. D., Denning, D. W., Gow, N. A. R., Levitz, S. M., Netea, M. G., & White, T. C. (2012). Hidden killers: Human fungal infections. Science Translational Medicine, Vol. 4. https://doi.org/10.1126/scitranslmed.3004404

[2] Chen, Y., Bruning, E., Rubino, J., & Eder, S. E. (2017). Role of female intimate hygiene in vulvovaginal health : Global hygiene practices and product usage. https://doi.org/10.1177/1745505717731011

[3] Donders, G. G. G., Ruban, K. S., Bellen, G., &Grinceviciene, S. (2018). Vulvovaginalcandidosis. Diagnostics to Pathogenomics of Sexually Transmitted Infections, pp. 293–327. https://doi.org/10.1002/9781119380924

[4] Eggimann, P., Garbino, J., &Pittet, D. (2003). Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. Lancet Infectious Diseases, Vol. 3, pp. 685– 702. https://doi.org/10.1016/S1473-3099(03)00801-6

[5] Ester, J., & Lopez, M. (2015). Women ' s health Candidiasis (vulvovaginal) Search date October 2013 DRUG TREATMENTS FOR ACUTE SYMPTOMATIC Women ' s health Candidiasis (vulvovaginal). (October 2013), 1–23.

[6] Fidel, P. L. (1998). Vaginal candidiasis: Review and role of local mucosal immunity. AIDS Patient Care and STDs, Vol. 12, pp. 359–366. https://doi.org/10.1089/apc.1998.12.359

[7] Global trends in the distribution of Candida species causing candidemia. (n.d.).

[8] Haya, J., García, A., López-manzanar, C., Balawi, M., &Haya, L. (2014). Importance of Lactic Acid in Maintaining Vaginal Health : A Review of Vaginitis and VaginosisEtiopathogenic Bases and a Proposal for a New Treatment. (September), 787–799.

[9] Nicole, W. (2014). A Question for Women's health: Chemicals in Feminine Hygiene products and personal Lubricants. Environmental Health Perspectives, 122(3). https://doi.org/10.1289/ehp.122-A70

[10] Rebecca Jeanmonod; Donald Jeanmonod. (2019). Vaginal Candidiasis (Vulvovaginal Candidiasis). NCBI.

[11] Shao, L. C., Sheng, C. Q., & Zhang, W. N. (2007). Recent advances in the study of

antifungal lead compounds with new chemical scaffolds. YaoxueXuebao, Vol. 42, pp. 1129–1136.

[12] Jeanmonod, R., &Jeanmonod, D. (2019). Vaginal Candidiasis (Vulvovaginal Candidiasis).In StatPearls [Internet].StatPearls Publishing.