



Research Article

FORMULATION AND EVALUATION OF VAGINAL DOUCHE MADE FROM ROBUSTA COFFEE (*Coffea canephora* L.) EXTRACT AND BETEL LEAF (*Piper betel* Linn) AGAINST *Candida albicans* FUNGUS GROWTH

Novi Fajar Utami¹, Oom Komala², Ria Komalasari¹, Akhmad Endang Zainal Hasan^{3*}

¹Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Pakuan University, Indonesia

²Department of Biology, Faculty of Mathematics and Natural Sciences, Pakuan University, Indonesia

³Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Indonesia

*Corresponding Author Email: pakzainalhasan@gmail.com

Article Received on: 29/10/18 Approved for publication: 22/11/18

DOI: 10.7897/2230-8407.0911256

ABSTRACT

Fluor albus is a problem that is very troubling for women. Abnormal *fluor albus* is characterized by a large amount of fluid, white in color such as stale milk, yellow or greenish, itchy, sore and accompanied by a fishy or rotten odor. The robusta coffee bean and betel leaf are plants that have been trusted their efficacy as anti-*fluor albus*. These plants have ability to inhibit *Candida albicans* growth. This study aims to determine the best formula of vaginal douche made from robusta bean extract and betel leaf. The method used in this research was dilution agar for MIC and diffusion of disc paper for LDH against formula 0 (base), formula 1 (Robusta extract 0.7%), formula 2 (betel extract 8%), formula 3 (combination 10%), formula 4 (combination 15%), formula 5 (combination 20%) and vaginal douche in the market as a positive control. The results showed that MIC in robusta extract was at concentrations of 0.5% and for betel leaf extract was at 0.6%. Whereas LDH results in formula 2 single betel leaf extract (8 gr / 100 ml) and formula 5 combination vaginal douche 20% (robusta 0.14 gr / 100 ml and betel leaf 1.6 gr / 100 ml) is the best vaginal douche formula as an antifungal of *Candida albicans*. Besides, this combination is better than the positive control of vaginal douche in the market.

Keywords: *Coffea canephora* L., *Piper betel* Linn, *Candida albicans*, Vaginal Douche.

INTRODUCTION

Fluor albus is a problem that is very troubling for women. *Fluor albus* is a discharge of liquid from the vagina other than menstrual blood¹. *Fluor albus* itself is a normal reproductive health problem and often occurs in women, especially teenagers. But keep in mind that *fluor albus* can also occur due to infections caused by bacteria, viruses, fungi or parasites². The cause of *fluor albus* is *Candida albicans* and is the most pathogenic candida species³. Prevalence and estimation of the number of cancer patients, cervical cancer is the highest cancer in Indonesia in 2013 which amounted to 0.8%⁴ and one of the main causes is *fluor albus*.

There are some plants in Indonesia that have been trusted as anti-*fluor albus* properties, one of them is robusta coffee beans and betel leaf. Robusta coffee beans (*Coffea canephora*) contain active substances that are efficacious such as alkaloids, flavonoids, saponins, tannins, caffeine and phenols⁵. The decoction of robusta coffee beans (*Coffea canephora* L) has the ability to inhibit *Candida albicans* with a minimum inhibitory concentration of 0.312%⁶.

Soap is a product that is often used by people as a laundry or skin cleanser. Various types of soap on the market vary widely, ranging from laundry soap, bath soap, hand soap, household cleaning soap in the form of creams, solids or bars, powder and liquid⁷. Liquid soap or in this case is vaginal douche especially is widely produced because of its practical and easy to use form. Not only that, liquid soap can also be used as a treatment, such as skin diseases due to fungi and bacteria.

Based on the chemical content and utilization of robusta coffee beans and betel leaves, research was conducted by formulating a combination of robusta coffee bean extract and betel leaf extract against the growth of *Candida albicans* as anti-*fluor albus*.

MATERIALS AND METHODS

Material Test. Robusta coffee beans and betel leaves were collected in March 2018 and identified by The Center for Plant Conservation from Bogor Botanical Garden with the authentic number is 803/IPH.1.01/If.07/III/2018. The specimen was deposited by Herbarium of Pharmacognosy Laboratorium Department of Pharmacy, University of Pakuan, Bogor, Indonesia (24/A/HLF/UNPAK).

Chemicals. Ethanol 96% (Sigma Chemical Co.), Sodium Lauryl Sulfate (*Brataco*®), NaCl (*Brataco*®), Propylene Glycol (*Brataco*®), Citric acid (*Brataco*®), aquadest, chloroform (*Merck*®), HCl (*Merck*®), Reagent Dragendorf, Reagent Mayer, Reagent Wagner, magnesium powder (*Brataco*®), FeCl₃ (*Merck*®).

Extraction. Simplicia powder of robusta coffee beans and betel leaves separately weighed 500 grams, extracted using maceration method with a solvent ratio of 1:10. The solvent used was 5 L of 96% ethanol. The filtrate results are dried with Rotary Vacuum Evaporator until it becomes a thick extract, which is then carried out again by quality testing including organoleptic, moisture content, ash content and phytochemical analysis.

MIC Test. Antifungal effectiveness testing is to determine the MIC using agar dilution methods. Ethanol extract concentration

96% of robusta coffee beans used 0.3%, 0.5%, 0.7%, 1%, 2%. While betel leaves extract with a concentration of 6%, 8%, 10%, 12%, 14%. By preparing 12 ml of liquid potato dextrose agar medium at 40 °C, then adding 0.2 ml of a suspension of *Candida albicans* mushroom spread over the surface of PDA media and adding 1 ml of robusta coffee bean extract from each concentration then homogeneous with how to move like the number eight. After that it was incubated at 37 °C for 24 hours.

The same treatment was carried out on betel leaf extract. After incubation, see and observe the presence of fungal colonies.

Vaginal Douche Formula. The Solution which has the lowest concentration that can inhibit the growth of *Candida albicans*, are used as active ingredients in vaginal douche formula ². The Formula of vaginal douche can be seen in Table 1.

Table 1: Vaginal Douche Formula

No	Composition	Formula (%b/v)					
		F0	F1	F2	F3	F4	F5
1	Robusta Extract	-	X	-	XY 10%	XY 15%	XY 20%
2	Betel Extract	-	-	Y			
3	Sodium Lauryl Sulphate	5	5	5	5	5	5
4	NaCl	5	5	5	5	5	5
5	Propyleneglycol	5	5	5	5	5	5
6	Citric acid	0.5	0.5	0.5	0.5	0.5	0.5
8	Aquadest	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

F0: base; F1: Formula Vaginal Douche robusta extract; F2: Formula Vaginal Douche betel leaf F3: Formula Vaginal Douche combination robusta 10 mL X and betel leaf 10 mL Y; F4: Formula Vaginal Douche combination robusta 15 mL X and betel leaf 15 mL F5: Formula Vaginal Douche combination robusta 20 mL X and betel leaf 20 mL.

Vaginal Douche Evaluation

1. Evaluation of Organoleptic. In the formulations that have been formulated, observations of appearance include odor, color, and texture of the preparation.

2. Evaluation of Homogeneity. Homogeneity test is carried out by means of each liquid soap formula weighed as 0.1 gram placed on the object glass, then observed.

3. Determination of pH value. PH measurements of the preparation are carried out using a pH meter. PH checking begins with pH calibration tool starting with a pH meter tool calibration using buffer pH 7 and pH 4. Putting pH meter into the soap solution that has been made, then wait until the pH meter indicator is stable and shows a constant pH.

4. Determination of Specific Weight. A clean and dry pycnometer is weighed. aquadest and liquid soap each inserted into the pycnometer using a dropper pipette. The pycnometer is closed, the volume of wasted liquid is cleaned by using a tissue and put into the cooler until the temperature is 25 °C. Then the pycnometer is allowed to stand at room temperature for 15 minutes and weighed the weight of the pycnometer containing liquid soap.

Inhibiting Power Width Test. The width of the inhibitory power is done using the disk diffusion method. Sterile potato dextrose liquid media (40 °C) mixed with 0.2 ml suspension of *Candida albicans* mushrooms then homogenized, let stand until the media becomes solid, after the media solidifies the disc paper has been soaked into the liquid soap formula extract of robusta coffee beans and betel leaf each formula 1, formula 2, formula 3, formula 4, formula 5, positive control (Vaginal Douche on the market) and negative formula 0 (base liquid soap without extract), then incubated at 37 °C for 24 hours . Furthermore, the width of the inhibition is measured as the clear area around the disc containing the test solution, positive control and negative control by using a caliper or ruler.

RESULT AND DISCUSSION

Robusta coffee used in this research was Robusta coffee which has been roasted and obtained from the shop in Bogor while the green betel leaf used was obtained from the Parungkuda-Sukabumi.

The characteristics of robusta coffee powder are that it has a bitter taste, a typical aromatic smell, brown color and a rough powder form. Green betel leaf powder has a bitter and spicy flavor, dark green color typical aromatic odor and fine powder form.

Robusta Coffee and Betel Leaf Thickened Extract

The extraction method used for this research was maceration. The maceration process is cheap and easy to do. The maceration process uses room temperature so as to minimize damage to bioactive compounds in the extract ⁹. The solvent used was 96% ethanol. The simplicia powder used was 500 grams with 96% 5 L ethanol solvent (1:10). The filtrate obtained is then dried using Rotary Vacuum Evaporator to reduce the content of the solvent contained. The extract obtained was a thick brown extract for the coffee and dark green betel leaves extract.

Minimum Inhibitory Concentration (MIC)

In order to determine the Minimum Inhibitory Concentration (MIC), it was made the robusta coffee thickened extract at the concentration of 0.3%; 0.5%; 0.7%; 1%; 2%. Concentration of extracts on betel leaves at 6%; 8%; 10%, 12%, 14%.

The lowest concentration (MIC) of robusta coffee bean extract and betel leaf which the growth of fungus did not occur in the petri was at concentrations of 0.5% for robusta coffee extract and 6% for betel leaf extract. The concentration was used as the initial reference for extract concentration in the manufacture of vaginal douche with an increase in concentration to 0.7% for robusta coffee extract and 8% for betel leaf extract.

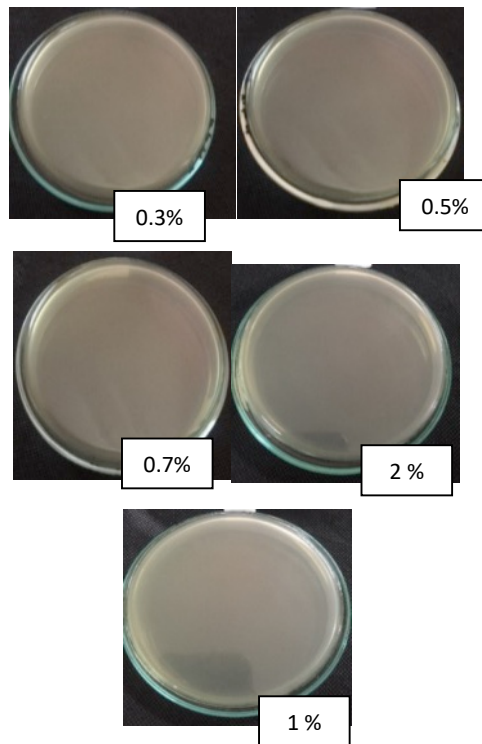


Fig 1. Result MIC robusta extract at 0.5%

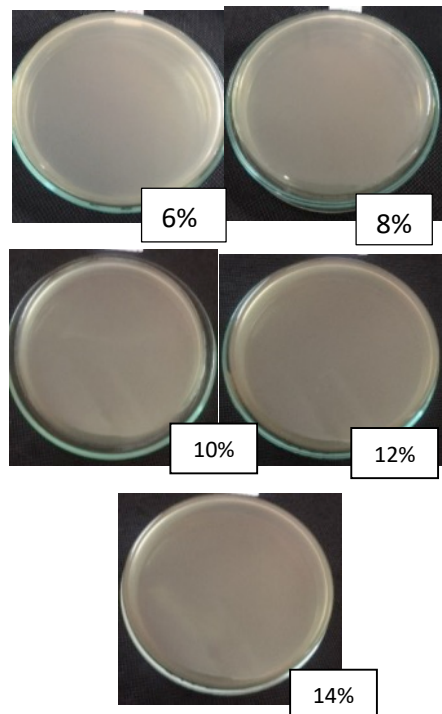


Fig 2. Result MIC betel leaf at 6%

Evaluation of Vaginal Douche Formula

The Organoleptic evaluation consists of the smell, color and dosage form. The results of organoleptic evaluation can be seen in table 2. The color of product from Formula 1, 2, 3, 4, 5 has the color of brown, dark green, light brown, brown to dark brown and a distinctive aroma. This difference is influenced by the addition of a single extract or a combination of robusta coffee and betel leaf. The color of the clear 0 (base) formula is colorless because the ingredients for the base used are colorless and there is no addition of extract. The dosage form of the vaginal douche formula is liquid.

The results of the homogeneity of vaginal douche evaluation found that vaginal douche made from robusta coffee bean extract and betel leaf was not all homogeneous. In formula 1 (coffee extract), formula 2 (betel leaf extract) still have deposited in the dosage. Due to the active substance used in the form of thick extract, the extract is not completely dissolved in water solvent. The time of dispersion and redispersion in the precipitate in vaginal douche which formed is calculated. The average vaginal douche dispersion time is more than 30 minutes and the vaginal douche formula can be easily redispersed. The criteria for a good

suspension or sediment are to be able to accurately settle slowly and easily flatten when shaken ¹⁰.

The test results of robusta coffee vaginal douche and betel leaf pH on base (F0) were 4.129; pH 3.949 on formula 1; pH 4.439 on formula 2; pH 4.351 on formula 3; pH 4.039 on formula 4; pH 4.712 on formula 5; while the pH for positive control is 5.496. The pH requirements of vaginal douche formula according to the Indonesian National Standard are 6-8. The pH reduction can be caused by the acidic nature of the coffee itself, the pH value in robusta coffee steeping is 5.61 ¹¹. In addition, a decrease in the pH value can also be caused by ingredients of soap which are acidic citric acid ¹².

Based on the Indonesian National Standard, the requirements for the specific gravity are 1.01 - 1.10. The results of determining the specific gravity of F0 (base) are 1.0381, F1 = 1.0370, F2 = 1.0369, F3 = 1.0054, F4 = 1.0039, F5 = 1.0034. As a test of the specific gravity of formula preparation, the vaginal douche was compared with the positive controls in the market with a specific gravity and the value which was obtained is 0.9930, it is not much different and meets the requirements.

Table 2: The Result of formula vaginal douche robusta extract and betel leaf

Evaluation		Formula 0	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Control positive
Organo-leptic	color	clear	Brown	Dark green	Light brown	brown	Dark brown	clear
	form	liquid	Liquid	liquid	liquid	Liquid	liquid	liquid
	Fragrance	odorless	coffee	betel	typical	Typical	typical	typical
Homogeneous		Homogeneous	Not Homogeneous	Not Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH		4.129	3.949	4.439	4.351	4.039	4.712	5.496
Specific Weight		1.0389	1.0370	1.0369	1.0054	1.0039	1.0034	0.9930

Inhibitory Power Width Test

The inhibitory value of antifungal compounds in robusta coffee bean extract, betel leaf extract and a combination of both extracts was carried out using a disc diffusion method for 7 treatments and 3 replications.

The data in table 3 shows that each treatment has an antifungal activity but with different inhibitory and category values. The width of the inhibitory power that was closest to the positive control was seen in formula 1 (Robusta coffee bean extract 0.7%). It was based on the results of SPSS 17.0 analysis which showed that there is no significant difference, or it has the same effect on

the positive control of vaginal douche on the market but with the inhibitory power which is categorized as a weak category. Whereas for formula 2 (8% betel extract) showed no significant difference or has the same effect as the formula 1, formula 3 (combination of 10%) and formula 4 (combination of 15%) where the inhibitory power includes in the medium category. Then in formula 5 (combination of 20%), it also showed no significant difference or the same effect with formula 2, formula 3, formula 4 where the inhibitory power is the medium category. Although formula 5 is not as influential as the positive control of vaginal douche on the market, however it has the highest width of inhibitory that is 6.69mm.

Table 3: Result Inhibitory Power Width Test vaginal douche robusta bean and betel leaf

Formula	Inhibitory Power Width (mm)	Category
Control positive	3.01 ^b	Weak
Formula 1 (robusta extract 0.7%)	4.496 ^{b,c}	Weak
Formula 2 (betel extract 8%)	5.506 ^{c,d}	medium
Formula 3 (combination 10%)	5.8 ^{c,d}	medium
Formula 4 (combination 15%)	5.85 ^{c,d}	medium
Formula 5 (combination 20%)	6.69 ^d	medium

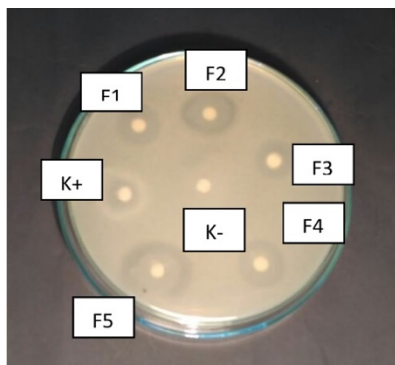


Fig 3: Inhibitory Power Width Test
 K+= control positive (vaginal douche on market)
 K- = control negative (base F0)
 F1= robusta extract 0,7%
 F2 = betel leaf 8%
 F3= combination 10%
 F4 = combination 15%
 F5 = combination 20%

Based on data analysis in Tables 1 and 3, it can be said that vaginal douche combined with robusta coffee bean extract and betel leaf extract can improve the antifungal effect and work synergistically between robusta coffee extract and betel leaf against *Candida albicans*.

Robusta coffee has alkaloid, flavonoid, saponin, tannin, caffeine and phenol compounds⁵. In damaging bacterial cells, flavonoids make use of the differences in polarity between lipid composing bacterial cells and alcohol groups on flavonoid compounds. While in the case of alkaloid compounds use the nature of the reactive base group in alkaloid compounds to react with amino acid groups in bacterial cells¹³⁻¹⁴.

The presence of phenols and kavikols in betel leaves is a toxic compound that results in disruption of the three-dimensional structure of the protein and opens into a random structure without any damage to the structure of the covalent framework. This causes denatured proteins. After denaturing the protein amino acid series remains intact, but its biological activity becomes damaged so that the proteins contained in the microbes cannot function¹⁵.

CONCLUSION

Based on the results of the study, it can be concluded that the combination of robusta coffee extract and betel leaf is more effective than each single extract. The best vaginal douche formulation as anti-fungal *Candida albicans* is formula 5 and is better than the positive control of vaginal douche on the market.

ACKNOWLEDGMENT

We are grateful to the Pakuan University Research and Development for financial (LPPM) for financial support.

REFERENCES

1. Kasdu, Dini. 2005. *Solution and Problem of Women*. Jakarta : Puspa Swara.
2. Mutmainah, Yuvianti Dwi Franyoto. 2015. Formulation and Evaluation of Liquid Soap Ethanol Extract of Red Ginger (*Zingiber Officinale Var Rubrum*) and Activity as Flour albus. *J Clinical Pharmacy & Pharmacy*. Vol 12. (1).
3. Dewi, Sri Sinto dan Aryadi, Tulus. 2010. Effectiveness of Virgin Coconut Oil (VCO) on In Vitro Candidiasis. *J Pharmacy proceedings*. Muhamadiyah University Semarang.
4. Ministry of Health. 2013. *Basic Health Research Data. Research and Development Agency of the Republic of Indonesia Ministry of Health and Target Population Data*, Pusdatin Ministry of Health of the Republic of Indonesia.
5. Yowanda, I. 2015. Comparison of the Inhibition of Robusta (*Coffea canephora*) and Arabica (*Coffea arabica*) Coffee on the Growth of *Candida albicans*. *Essay*. Aceh : Syiah Kuala University.
6. Nugraha, Ahmad., Suwendar, Siti Hazar. 2016. Anti-Microbial Potential of Decoction of Robusta (*Coffea canephora* L) Coffee Beans against *Staphylococcus aureus*, *Escheria Coli*, and *Candida albicans*. *Pharmacy proceedings*. Vol. 2 (2).
7. Ari, Wibisana. dan Budiyo. 2004. *Formulation Liquid Soap with Basic Ingredients Alkyl Benzene Sulfonate*. (<http://www.angelfire.com>, accessed on Februari 2007).
8. Prameswari, Okky Meidiana., Simon Bambang Widjanarko. 2014. The Effect of Water Extract of Pandan Wangi Leaf to Decrease Blood Glucose Levels and Pancreas Histopathology at Diabetes Mellitus Rats. *J food and industrial products* Vol 2:2
9. Ansel, Howard C., Loyd V. Allen, Jr., Nicholas G. Popovich., 1993. *Pharmaceutical Dosage Forms and Drugs Delivery Systems. Seventh Edition*. United States of America
10. Aditya, I Wayan. 2015. Caffeine Content of Coffee Powder pH Value and Characteristics of Taste and Aroma of steeping Male Coffee (Pea berry coffee) and Females (flat beans coffee) Types of Arabica and Robusta. *Essay*. Udayana University.
11. Apriani, D., 2013. Formulation of Liquid Bath Soap for Essential Oil of Lime (*Citrus aurantifolia*) with Cocamide DEA as a surfactant. *Essay*. Muhamadiyah University Surakarta.
12. Gunawan, I.W.A. (2009). *Potential Pare (Momordica Charantia L) As Antibacterial Salmonella typhimurium*. Denpasar: Department biology Program Studi Pendidikan Biologi Faculty of Teacher Training and Education. Mahasaraswati University
13. Hasan AEZ . 2013. Induction resistance of *Candida* sp. Y 390 to ethanol stress by kopyor coconut and virgin coconut oil . *Emir. J. Food Agric*. 25 (10): 790-797
14. Mudatsir, susanti, hafnati. 2007, Antimicrobial Activity Test of Methanol Extract of Betel Leaves (*Piper betel* L) Against *Candida albicans* in In Vitro. *J Syiah Kuala Doc*, 7(3):117-130.

Cite this article as:

Novi Fajar Utami et al. Formulation and evaluation of vaginal douche made from Robusta coffee (*Coffea canephora* L.) extract and Betel leaf (*Piper betel* Linn) against *Candida albicans* fungus growth. *Int. Res. J. Pharm.* 2018;9(11):48-52 <http://dx.doi.org/10.7897/2230-8407.0911256>

Source of support: Pakuan University Research and Development, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.