SAINSTEK: JURNAL SAINS DAN TEKNOLOGI
Publisher: AMSET IAIN Batusangkar and IAIN Batusangkar Press
Website: http://ecampus.iainbatusangkar.ac.id/ojs/index.php/sainstek
E-mail: sainstek@iainbatusangkar.ac.id

December 2021
Vol 13 No 2
ISSN: 2085-8019 (p)
ISSN: 2580-278X (e)
pp : 105-111

Methanol Extracts Formulation of Tambora Leaves (*Ageratum conyzoides* L.), Sembalit Angin Leaves (*Mussaendafrondosa* L.) and Turmina Rhizome (*Curcuma longa*) as *Candida albicans* Antifungal

Bunga Indah[1], Noor Hujjatusnaini[1]*, Astuti Muh. Amin[2], Lilin Ika Nur Indahsari[1]

[1]*Departement of Biology Education, FTIK, IAIN Palangka Raya, Central Kalimantan, Indonesia.*
[2]*Departement of Biology Education, FTIK, IAIN Ternate,Ternate, North Maluku, Indonesia.*
**email: noor.hujjatusnaini@iain-palangkaraya.ac.id*

Article History
Received: 29 May 2021
Reviewed: 9 June 2021
Accepted: 22 October 2021
Published: 20 December 2021

Key Words
Combination formula;
Ageratum conyzoides;
Mussaendafrondosa;
Curcuma longa;
Candidaalbicans.

Abstract
This study aimed to analyze the combination formula of Tambora leaves, Sembalit Angin leaves, and Turmeric Rhizomes using a 2: 1: 3 ratio, and determine its effect on the growth of *Candida albicans*. The growth indicator was measured based on the growth area of inhibition zone which marked by a clear zone between the outer sides of the paper disc. The growth activity was measured at the incubation period of 1x24 hours, 2x24 hours, and 3x24 hours. The observation data then were analyzed by oneway ANOVA statistical test continued with the Duncan test 1%. The results showed that the combination formula of bioherbal methanol extract of Tambora leaves, Sembalit Angin leaves, and turmeric rhizomes had a significant effect on the growth of *Candida albicans*at 1% significance, proven by the sig value. 0.00 <0.01. Therefore, the combination formula of bioherbal methanol extract of Tambora leaves, Sembalit Angin leaves, and Turmeric rhizomes can be recommended as a combination formula extract that can inhibit the growth of *Candida albicans*.

INTRODUCTION

Plants have an important role for human survival, specifically their potency as herbal medicine (Mingga et al., 2019). According to (Fuadi, 2017), Indonesia has about 30,000 plants species, which 80% of them were used by the community for herbal medicine. Central Kalimantan has about 20,000 species of medicinal plants (Zuhud, 2009), such as the form of shrubs (Hidayat & Hardiansyah, 2012), tubers (Ngurah et al., 2016), epiphytic plants (Gunadi et al., 2017), or higher plants (Umar, 2017). Commonly, this plant is believed to be used as traditional medicine. The richness of medicinal plant species in Central Kalimantan is part of the biodiversity that must be maintained. Nevertheless, the wealth of medicinal plants in Central Kalimantan is not comparable to efforts to record scientific information related to the diversity of plant species. The lack of information and public knowledge about medicinal plants indicates the need for further study. Efforts to research and publish about natural wealth especially in herbal medicine is needed in order that the information will be collected and give the wider benefits.The use of bioherbs or medicinal plants in Central Kalimantan is part of the natural wealth that is closely related to culture from generation to generation (Ani et al., 2018), which is generally conducted by mixing (Gunadi et al., 2017), or by combining several plant species (Ningsih, 2016). The use of a

combination of several plants is traditionally believed to have medicinal properties against infections, one of which is postpartum infection. However, the combination of this plant as a medicinal ingredient is still unrecognized and scientifically proven. Therefore, it is still necessary to prove the correct combination formula of medicinal plants against postpartum infections. Several previous studies report that postpartum infections are generally caused by groups of microorganisms including *Candida albicans* (Yanti et al., 2016).

Tambora leaves are reported to have potential as antibacterial and wound healers because it contains flavonoids, saponins, and tannins (Astuti, 2015). The chemical content of the plant also has the potential as antifungal because the compounds contained in the leaves have antimicrobial properties (In-vitro & Febia, 2020). Sembalit Angin plant according to (Garvita, 2015) also contains alkaloids, saponins, glycosides, flavonoids, and tannins. These secondary metabolites are also found in turmeric rhizomes. (Pangemanan et al., 2016) explained that curcumin and essential oil compounds in turmeric have antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. This is in line with the statement of (Kusbiantoro & Purwaningrum, 2018) that the turmeric rhizome has antimicrobial and antifungal activity in inhibiting fungal growth, one of which is *Candida albicans*.

Halimatussa'diah et al., (2014) reported that the use of plants combination in medicine has more effective healing effect than single plant. The combination of medicinal plants in certain scientific formula will have a synergistic effect and complement each other's potential against microorganisms cause infection. The final finding of this research is an available information about the correct formulation of the bioherbal combination for *Candida albicans*.

METHOD

Tools and Materials

Research tools include autoclave, 1000 mm becker glass, 500 mm becker glass, 250 mm becker glass, 100 mm becker glass, 50 mm becker glass, evaporator, test tube, 500 mm erlenmeyer flask, 250 mm erlenmeyer flask, petri dish, jam glass. , inoculating needle, iron stirrer, glass stirrer, glass funnel, tweezers, magnetic stirrer, micropipette, pipette, LAF, hot plate, incubator, digital balance, weighing scale, scissors, cutter, bunsen lamp, blender, basin, tray, gas stove, caliper, pans, stationery, napkins, refrigerator, cotton buds, and aluminum foil. Research materials include Tambora leaves, Sembalit Angin leves, Turmeric rhizome, pure culture of *Candida albicans* strain 33114, SDA medium, beef extract, becto peptone, aquadest, 70% alcohol, methanol, cotton, vaseline, filter paper, cover paper, gauze, label paper, blotting paper, rubber band, lysol, and laundry soap.

Extract Combination Formula Preparation

The preparation of the extract combination formula was designed in a 2:1:3 formula. The master stock of the extract was then made in the determined research concentration level. The positive control of the study used Albothyl 0.25% and Aquades as the negative control of the study.

The stages of the research were: (1) plant sampling is carried out in the Palangka Raya City area. (2) powder making into simplicia takes several days since drying the medicinal plant samples then grind them into powder, (3) extraction is the process of separating a component from the mixture by using a number of solvents as a separator. (4) extract testing with a 2:1:3 combination formula with a concentration level of 30%, 40%, 50%, 60%, 70%, and 80%, the test is based on previous research and based on people's habits. The research was conducted at the Microbiology Laboratory of IAIN Palangka Raya, Central Kalimantan. The measurement of inhibition was seen from the clear zone between the *Candida albicans* colony and the outer side of the paper disc containing the extract of SDA (Sabouraud Dextrose Agar) medium.

Analysis of Antifungal Combination

Analysis of extracts against *Candida albicans* was previously carried out by culturing *Candida albicans* in liquid medium and incubated for 1x24 hours. after that it was planted in solid SDA using 1 ose in 10 petri dishes**.** Then the disc paper was soaked for 1 minute into each concentration level. Paper disc

wasthen placed in the center of the surface of the SDA medium, and all of them were incubated at 37°C. Observation of *Candida albicans* growth inhibition was conducted at each incubation time.

Data Analysis

Observational data was carried out after giving treatment with 4 replications and the Data from the observations were then analyzed with the one-way ANOVA statistical test and continued with Duncan's 1% test to determine the significance.

RESULTS AND DISCUSSION

Observation of the growth inhibition zone of *Candida albicans* was carried out on all formulations of the treatment samples during the designed incubation period. Data from observations of the growth inhibition zone of *Candida albicans* on SDA medium was shown in Table 1.

The recapitulation data in Table 1 has an average inhibition zone that varied between 1.90 mm (Albothyl 0.25%) to 2.26 mm (P4=40%) for a 24-hour observation time, 1.86 mm (Albothyl 0.25%) to 3.88 mm. (P6=60%) for the observation time of 48 hours, and 2.10 mm (Albothyl 0.25%) to 5.53 mm (P8=80%) for the observation time of 72 hours. This showed that the growth inhibition at certain concentrations was stronger than the positive control of the research.

To determine the significance of the influence of variables in the study, the ANOVA statistical test was carried out, the results of which are presented in Table 2. The calculation of the analysis results in Table 2 shows that the combination formula of 2:1:3 has a significant effect, confirmed by by the Sig value 0.000 < 0.01, both in the entire incubation period. These data were strengthened by the comparison of the mean square values presented in Figure 1

Table 1. Recapitulation of Candida Albicans. Growth Inhibitory Zones

Extract Combination formula 2:1:3	Average Inhibiton Zone (mm)		
	24 Hours	48 Hours	72 Hours
Albothyl 0.25%(+)	1.90	1.86	2.10
Aquades (-)	0	0	0
30%	1.40	2.77	3.39
40%	2.26	3.64	4.73
50%	1.76	3.38	4.51
60%	1.31	3.88	3.30
70%	1.66	2.29	2.64
80%	1.93	1.93	5.53

Table 2. The Significance of the Influence of Variables Using ANOVA Statistical Test

	24 hours		48 Hours		72 Hours	
	Sum of Squares	Sig.	Sum of Squares	Sig.	Sum of Squares	Sig.
Between Groups	13.186	,000	44.274	,000	84.962	,000
Within Groups	9.937		10.809		21.866	
Total	23.123		55.083		106.828	

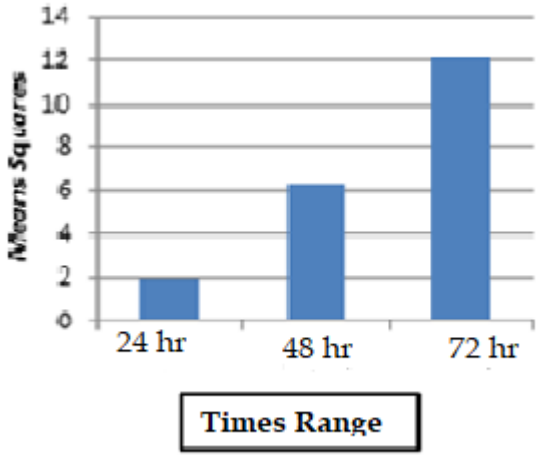

Figure 1. Mean Square Formula 2:1:3 Combination Against the Growth Inhibition Zone of *Candida albicans*

Table 3. Duncan's Test Results 1% Inhibition Zone for Candida albicans Growth Combination 2:1:3 at 24 Hours

Combination formula	N	Notasi Subset for alpha = 0.01	
		1	2
P2	4	0 a	
P6	4		1.31 b
P3	4		1.40 b
P7	4		1.66 b
P5	4		1.76 b
P1	4		1.90 b
P8	4		1.93 b
P4	4		2.26 b
Sig.		1.000	.080

The significance value of the 2:1:3 extract combination formula was confirmed by the mean square ratio in Figure 1. The mean square comparison showed the fact that the 72 hours incubation period had a much greater difference in the variable effect optimization compared to 24 hours and 48 hours.Therefore, the results could be be used as an indicator to determine the most effective concentration formula. The 2:1:3 combination formula of bioherbal extracts had a significant effect on the growth inhibition zone of *Candida albicans* at a significance of 1% (Table 2).

The significance of the incubation period for the observation of the *Candida albicans* growth inhibition zone was confirmed by Table 3, Table 4, and Table 5.

Table 3 showed the data on all treatment extract concentrations in the 2:1:3 combination formula which had the same inhibitory power between all concentration levels and did not differ significantly from the positive control (P1), so that the concentration was interpreted as having the same ability as Albothyl 0.25%. The smallest concentration in this study was the concentration of 30% which had an average inhibition zone that did not differ from the larger concentration, so the concentration of 30% was defined as the effective concentration of the study. Observations at the incubation period of 48 hours (see Table 4).

Table 4. Duncan Test Results 1% Inhibition Zone for *Candida albicans* Growth Combination 2:1:3 at 48 Hours

Combination formula	N	Notasi Subset for alpha = 0.01			
		1	2	3	4
P2	4	0 a			
P1	4		1.86 b		
P8	4		1.93 b		
P7	4		2.29 b	2.29 c	
P3	4		2.77 b	2.77 c	2.77 d
P5	4			3.38 c	3.38 d
P4	4			3.64 c	3.64 d
P6	4				3.88 d
Sig.		1.000	.093	.014	.039

Table 5. Duncan Test Results 1% Growth Inhibition Zone *Candida albicans* Combination 2:1:3 at 72 Hours

Combination formula	N	Notasi Subset for alpha = 0.01				
		1	2	3	4	5
P2	4	0 a				
P1	4		2.10 b			
P7	4		2.64 b	2.64 c		
P6	4		3.30 b	3.30 c	3.30 d	
P3	4		3.39 b	3.39 c	3.39 d	
P5	4			4.51 c	4.51 d	4.51 e
P4	4				4.73 d	4.73 e
P8	4					5.53 e
Sig.		1.000	.092	.017	.062	.165

The treatment effect on the observation time during 1x24 hours and 2x24 hours had almost the same interpretation. The results of Duncan's 1% test showed that the concentration levels of P3, P6, and P7 were not significantly different from P1, so the concentration was interpreted to have the same ability as Albothyl 0.25%. However, these concentrations were significantly different when compared to P4 and P5.

The concentration level of P3 (30%) as the minimum concentration had the same inhibitory power as all concentration levels, except in concentration of 80%. Therefore, the concentration of 30% can be expressed as the effective concentration and the concentration of 80% was stated as the optimum concentration in inhibiting the growth of *Candida albicans* with an incubation period of 48 hours.

The effectiveness of secondary metabolites in a 2:1:3 combination formula was then observed during an incubation period of 72 hours, aimed to confirm the results of previous observations, and to know the inhibitory ability of fungi contained in secondary metabolites in natural ingredients contained in combination formula.

Table 5 illustrated a very significant decrease in inhibitory ability, proven by the notation that was not different from several concentrations that had low inhibitory power, such as P6 and P7. However, P3 (30%) was not significantly different from other concentration levels, except for the 80% concentration level. This empirical data show that the effectiveness is still present at a minimum concentration of 30%. The results of this statistical analysis reported that high concentration was not necessarily in line with the optimum inhibitory power possessed by natural substances in vitro.

The extracts combination formula with a ratio of 2:1:3 contained turmeric rhizome as the major composition. The appropriate compotition was about 50% turmeric rhizome extract, 30% Tambora leaf extract, and 20% Sembalit Angin leaf extract. According to

(Hariyati et al., 2015) the content in turmeric is flavonoids, tannins, alkaloids, essential oils and curcumin. (Azizah & Salamah, 2013) state that these compounds are known to have antiviral and antifungal activity. (Suraini & Putri, 2018) confirms that turmeric contains antimicrobials, including *Candida albicans*. Turmeric contains essential oils and curcumin which have been shown to have antioxidant and anti-inflammatory properties. Inflammation in tissues or organ systems can be caused by pathogenic microorganisms. The findings of this study are in line with research (Ardiansyah et al., 2021) that extract combination formula 3:2:1 of Tambora leaf, Sembalit Angin leaf, Turmeric rhizome have better potential when combined with other bioactives as antimicrobials. This finding confirms its potential as an antifungal in this study. Therefore, the findings from this study strengthened the findings of previous studies, when Turmeric rhizome was used in a dominant combination in a 2:1:3 formulation shown to have growth inhibition of *Candida albicans*, and can be recommended as antifungal combination formula.

CONCLUSION

Based on the results of the analysis showed tha the combination of 2:1:3 can inhibit the growth of *Candida albicans*. Combination formula of bioherbal methanol extract 2:1:3 with concentration at 30% level had a significant effect on *Candida albicans*.

REFERENCES

Ani, N., Rohyani, I. S., Program, M. U., Biologi, S., Matematika, F., Ilmu, D., & Alam, P. (2018). Pengetahuan Masyarakat Tentang Jenis Tumbuhan Obat Di Kawasan Taman Wisata Alam Madapangga Sumbawa. *Jurnal Pijar Mipa*, *13*(2), 160–166. https://doi.org/10.29303/JPM.V13I2.751

Ardiansyah, Hujjatusnaini, N., Amin, A. M., & Indahsari, L. I. Nu. (2021). Antibacterial Effectiveness of Methanol Extract Combination Formula 3:2:1 of Tambora Leaf (Ageratum conyzoides), Sembalit Angin Leaf (Mussaenda frondosa L), Turmeric Rhizome (Curcuma longa L) on the growth of Staphylococcos aureus. *Sainstek : Jurnal Sains Dan Teknologi*, *13*(1), 1–6. https://doi.org/10.31958/JS.V13I1.3513

Astuti, H. (2015). Uji Aktivitas Antibakteri Ekstrak Etanol Dan Ekstrak Air Daun Bandotan (Ageratum Conyzoides , L .) Terhadap Staphylococcus aureus Dan Escherichia coli. *Majalah Farnaseutik*, *11*(1), 290–293.

Azizah, B., & Salamah, N. (2013). Standarisasi Parameter Non Spesifik Dan Perbandingan Kadar Kurkumin Ekstrak Etanol Dan Ekstrak Terpurifikasi Rimpang Kunyit. *Pharmaciana*, *3*(1). https://doi.org/10.12928/pharmaciana.v3i1.416

Fuadi, T. M. (2017). Etnobotani Dan Identifikasi Tumbuhan Obat Bagi Ibu Pasca Melahirkan Di Desa Krueng Kluet Kecamatan Kluet Utara Aceh Selatan. *Prosiding Biotik*, *5*(1), 280–288. https://jurnal.ar-raniry.ac.id/index.php/PBiotik/article/view/2161

Garvita, R. V. (2015). Pemanfaatan Tumbuhan Obat Secara Tradisional untuk Memperlancar Persalinan oleh Suku Dayak Meratus di Kalimantan Selatan. *Warta Kebun Raya*, *13*(2), 51–58.

Gunadi, D., Oramahi, H. ., & Tavita, G. E. (2017). Studi tumbuhan obat pada etnis dayak di desa gerantung Kecamatan Monterado Kabupaten Bengkayang. *Jurnal Hutan Lestari*, *5*(2), 425–436.

Halimatussa'diah, F., Fitriani, V. Y., & Rijai, L. (2014). *Aktivitas Antioksidan Kombinasi Daun Cempedak (Artocarpus Champedan) Dan Daun Bandotan (Ageratum Conyzoides L) Fauziah*. *2*(5), 248–251.

Hariyati, T., Jekti, D. S. D., & Andayani, Y. (2015). Pengaruh Ekstrak Etanol Daun Jambu Air (Syzygium Aqueum) Terhadap Bakteri Isolat Klinis Titi. *Journal Penelitian Pendidikan IPA*, *1*(2), 31–38.

Hidayat, D., & Hardiansyah, G. (2012). Studi Keanekaragaman Jenis Tumbuhan Obat di Kawasan IUPHHK PT . Sari Bumi Kusuma Camp Tontang Kabupaten Sintang. *Jurnal Vokasi*, *8*, 61–68.

In-vitro, I. S., & Febia, A. (2020). *Aktivitas Antifungi Ekstrak Metanol Daun Bandotan (Ageratum conyzoides) Terhadap Phytophthora sp. 9*, 167–174.

Kusbiantoro, D., & Purwaningrum, Y. (2018). *Pemanfaatan Kandungan Metabolit Sekunder Pada Tanaman Kunyit Dalam Mendukung Peningkatan Pendapatan Masyarakat Utilization Of Secondary Metabolite In The Turmeric Plant To Increase Community Income*. *17*(1), 544–549.

Mingga, M., Mingga, M., Oramahi, H. A., & Tavita, G. E. (2019). Pemanfaatan Tumbuhan Obat Oleh Masyarakat Di Desa Raba Kecamatan Menjalin Kabupaten Landak. *JURNAL HUTAN LESTARI*, *7*(1), 97–105. https://doi.org/10.26418/jhl.v7i1.31005

Ngurah, I. G., Putra, P., & Estiasih, T. (2016). *Potensi Hepatoprotektor Umbi-Umbian Lokal Inferior : Kajian Pustaka Hepatoprotective Potential of Local Inferior Tubers : A Review*. *4*(1), 436–442.

Ningsih, I. Y. (2016). Studi Etnofarmasi Penggunaan Tumbuhan Obat Oleh Suku Tengger Di Kabupaten Lumajang Dan Malang, Jawa Timur. *Pharmachy*, *13*(01), 10.

Pangemanan, A., . F., & Budiarso, F. (2016). Uji daya hambat ekstrak rimpang kunyit (Curcuma longa) terhadap pertumbuhan bakteri Staphylococcus aureus dan Pseudomonas sp. *Jurnal E-Biomedik*, *4*(1). https://doi.org/10.35790/ebm.4.1.2016.10840

Suraini, & Putri, N. (2018). *EFEKTIFITAS ANTI JAMUR CAMPURAN REBUSAN JAHE (Zingiber officinale) DAN KUNYIT (Curcuma domestica) TERHADAP PERTUMBUHAN Candida albicans*. *1*(2), 73–77.

Umar, U. Z. (2017). *Analisis Vegetasi Angiospermae di Taman Wisata Wira Garden Lampung,*. 61–62.

Yanti, N., Samingan, & Mudatsir. (2016). Uji Aktivitas Antifungi Ekstrak Etanol Gal Manjakani (Quercus Infectoria) terhadap Candida albicans. *Angewandte Chemie International Edition*, *1*(1), 1–9.

Zuhud, E. A. M. (2009). Potensi Hutan Tropika Indonesia sebagai Penyangga Bahan Obat Alam untuk Kesehatan Bangsa. *Jurnal Bahan Alam Indonesia*, *6*(6).