Antifungal effect of ethyl acetate fraction of *Sarang semut* (*Myrmecodia pendens Merr.& Perry*) against *Candida albicanS*

Felisha Febriane Balafif[1*], Mieke Hemiawati Satari[1], Diah Dhianawaty[2]

[1*]Department of Oral Biology, Faculty of Dentistry Universitas Padjadjaran, Indonesia
[2]Department of Biochemistry, Faculty of Medicine Universitas Padjadjaran, Indonesia

ABSTRACT

Introduction: Oral candidiasis is a fungal infection of the oral cavity that is caused by *Candida albicans* (*C. albicans*). Treatment of oral candidiasis usually uses topical agents such as nystatin, but it comes with side effects. Research on medicinal materials from plants can be an alternative to chemical drugs. It has increasingly become a concern such as the tuber of *sarang semut* (*Myrmecodia pendens* Merr. & L.M. Perry). The aim of the study is to determine the effect of ethyl acetate and hexane fractions of *M. pendens* against *C. albicans*. **Methods:** *M. pendens* methanol extract used maceration method. As much as 33 g concentrated *M. pendens* methanol extract was partitioned with a separating funnel based on the polarity of the substances to obtain ethyl acetate fraction. The minimum inhibitory concentration (MIC) procedure was carried out by the microdilution method and measured by an ELISA reader. The minimum fungicidal concentration (MFC) was obtained conclusively on Mueller Hinton agar media at concentrations above MIC of ethyl acetate fraction of *M. pendens*. **Results:** Ethyl acetate fraction had antifungal effect against *C. albicans*. The MIC and MFC of ethyl acetate fraction were 625 µg/ml and 1.250 µg/ml. **Conclusion:** *Ethyl acetate* fraction of *M. pendens* can inhibit the growth of *C. albicans*. *M. pendens* can be developed as the prevention agent of oral candidiasis.

Keywords: antifungal; *candida albicans;* ethyl acetate fraction; *Myrmecodia pendens*

p-ISSN: 1979-0201; e-ISSN: 2549-6212; Available from: http://jurnal.unpad.ac.id/pjd/article/view/36703
DOI: 10.24198/pjd.vol34no3.36703
Submission: Nov 19, 2021; Accepted: Nov 30, 2022; Published online: Nov 30, 2022

INTRODUCTION

The oral microbiota is one of the most complex and diverse microbial communities in the human body. Balance between the human host and the intrinsic microorganisms is essential to maintain oral health. The healthy oral cavity is represented by a great microbial diversity, including both bacteria and fungi. One of the fungi in the oral cavity is candida. Candida can be found in the normal oral flora of a healthy individual. It is estimated that 45-65% of healthy infants and 30-55% of healthy adults. When there is an imbalance of normal flora of oral cavity, it causes overgrowth

**Corresponding author: Felisha Febriane Balafif. Department of Oral Biology, Faculty of Dentistry Universitas Padjadjaran, Indonesia. Jalan Sekeloa Selatan No. 1, Bandung, West Java, Indonesia 40132. Phone: +6282214620286; Email: felisha.balafif@unpad.ac.id*

of Candida. *C. albicans* that attack the oral cavity can cause a disease called candidiasis.[1, 2]

Various local and systemic factors lead to pathogenic Candida. Predisposing of local factors of oral candidiasis is poor oral hygiene, habit of night time wearing denture, daily total hours of wearing and denture or upper removable orthodontic appliance, impaired salivary gland function, oral steroid, and oral cancer. Predisposing of systemic factors is reduced immunity, diabetes, Cushing's syndrome, HIV infection, malignancies such as leukemia and nutritional deficiencies, and smoking.[3,4]

The treatment of oral candidiasis usually uses systemic and topical agents. A systemic antifungal agent is taken when infection spreads more widely. A topical antifungal agent is applied to the infected area and treats the limited area infected, such as nystatin. Nystatin is a membrane active polyene macrolide produced by *Streptomyces noursei* strains. However, it has side effects include metallic taste, dry mouth, anorexia and nausea.[5,6]

Research on herbal plants has increasingly become a concern for candidiasis treatment. The use of herbal products as topical antifungal agents is safe, effective, and not resistant. One of these plants is the tuber of *sarang semut (Myrmecodia pendens* Merr. & L.M. Perry). This epiphyte plant is the Tracheophyta division, *Magnoliopsida* class, *Lamiidae* subclass, *Rubiales* order, *Rubiaceae* family, and *Myrmecodia* genus. It contains several active compounds including flavonoid, steroid, terpenoid, and alkaloid that has antioxidant, anti inflammation, antibacterial, and antifungal effects.[7,8,9]

The tuber of *M. pendens* has activity as antifungal, antibacterial, anticancer, and antioxidant properties. *M. pendens* can be used as herbal remedies and the water boiling extraction can be employed as a simple manner for community herbal medicine without any toxic effect on cells. Moreover, infusion or ant nest boiled water contains antifungal substances that can inhibit growth *C. albicans*. Inhibition test of ant nest infusion using plastic containers stored at room temperature with a concentration of 20, 40, 60, 80,100% formed inhibition or halo area.[10,11]

Separation and grouping the chemical compounds of the extract based on the polarity is called fractionation. It has several solvents that are very influential on the extraction of active compounds, such as water, ethyl acetate, and hexane fraction. Previous research found that the content of water fraction compounds of this plant had an antifungal effect against *C. albicans*. Water fraction can inhibit growth of *C. albicans* with minimum concentration 1.250 µg/ml and kill growth of *C. albicans* with minimum concentration 2.500 µg/ml.[12]

Based on previous study, water fraction of *M. pendens* can kill and inhibit growth of C. albicans. However, whether ethyl acetate fraction of *M. pendens* can inhibit or kill *C. albicans* remains unknown. The purpose of this study is to determine the effect of ethyl acetate fraction of *M. pendens* against *C. albicans* ATCC 10231.

METHODS

The materials used were *C. albicans* ATCC 10231 (Microbiologics, Mi, USA), tuber of an *sarang semut* (*Myrmecodia pendens* Merr. & L.M. Perry), Mueller Hinton agar, Mueller Hinton broth, *saboraud dextrose agar* (SDA), dimethyl sulfoxide (DMSO), methanol, ethyl acetate, aquadest, chloroform-amoniac, sulfuric acid, HCl, and powder (Mg, $FeCl_3$). *M. pendens* was identified no. 099/HB/05/2015 by Herbarium Jatinangor, Taxonomy of Plant Laboratory, Department Biology, Faculty Mathematic and Natural Science, University of Padjadjaran.

M. pendens tuber *extract*

Manufacture *of M. pendens* methanol extract used maceration method. As many as 300 g of *M. pendens* tubers were cut into small pieces. It was extracted with 600 ml of methanol (MeOH) which was heated for 16 hours. The liquid methanol extract was evaporated with a rotary evaporator at 46°C to remove the solvent so that it produced a concentrated methanol extract *of M. pendens* tubers with a concentration of 100%.[14]

M. pendens tuber fraction

As much as 33 g concentrated extract was partitioned with a separating funnel based on the polarity of the substances contained in *M. pendens* extract tuber. At first, concentrated extract was placed in a separating funnel, then given 100 ml of

distilled water solvent and 100 ml of ethyl acetate solvent. It was shaken until everything dissolved, let stand for a moment, separated into 2 parts namely ethyl acetate solution at the top and water fraction at the bottom. The ethyl acetate solution was accommodated in erlenmeyer glasses, then it was put into the evaporator to get the concentrated ethyl acetate fraction of *M. pendens*. The compounds contained in ethyl acetate fraction were semipolar.[9,14]

Preparation of test fungi

Candida albicans ATCC 10231 was first rejuvenated by multiplying the fungi on the medium of Saboraud dextrose agar (SDA) and incubated for 18-24 hours at 37°C. One of these from the subculture was taken and suspended in 5 ml of Mueller Hinton liquid medium according to 0.5 McFarland standard.[15]

Test of the inhibitory of ethyl acetate fraction *of M.pendens* on *C. albicans* with diffusion agar method Kirby Bauer

As much as 10,000 µg of ethyl acetate of *M. pendens* fraction were mixed with 1 ml of 100% DMSO so that a concentration of 10,000 µg/ml was obtained. The ethyl acetate fraction of *M. pendens* was dissolved in DMSO with two-fold serial dilutions. A two-fold dilution is reduces the original concentration by one half. It obtains a concentration of 5,000 µg/ml and 2,500 µg/ml. Making the concentration of nystatin was carried out by adding 50 µg of nystatin into 1 ml of water to obtain a concentration of 50 µg/ml.

The inhibitory test of ethyl acetate fraction of *M. pendens* in *C. albicans* was carried out by pouring 28-30 ml of Mueller Hinton medium into a 100 mm diameter petri dish with a thickness of about 4 mm. Mueller Hinton was allowed to solidify. Suspension of *C. albicans* which was equivalent to turbidity 0.5 Mc Farland was taken as much as 0.1 ml and planted using a cotton bud on the agar plate. A total of 5 pieces of 8 mm diameter discs were dropped with 50 µl of ethyl acetate fraction of *M. pendens* with a concentration of 10,000 µg/ml, 5,000 µg/ml, and 2,500 µg/ml on the first, second and third paper discs, and then 50µl of nystatin were concentrated 50 µg/ml into the fourth paper disc as comparison. The fifth paper disc was dripped with 50 µl of 100% DMSO solvent. Each paper disc was placed on the surface of the agar medium, then incubated at 37°C for 24 hours. Repetition on this method was done twice. The diameter of the obstacle area was measured by calculating the diameter of the inhibition area minus the diameter of the disc.[16]

Determination of the minimum inhibitory concentration (MIC)

Procedure was carried out by serial microdilution method and measured by an ELISA reader to read the optical density of each well. The fraction was serially diluted in a 96-well microplate with DMSO as a medium. In short, 100 µl of the test sample was added to well column 1, and column 1 to column 12 initially received 100 µl of DMSO. Two-fold serial dilutions were made by transferring 100 µl from column 1 to column 2 and continued through column 12. The final concentration ranged from 2,44 µg/ml to 5000 µg/ml. The fungal suspension 10 µl was added to the well, and then the plate was incubated for 24 h at 37◦C. The determination of MIC was measured by calculating the percentage of fungal inhibition which can be calculated by the following formula. The percentage inhibition was calculated using the following formula: [(OD growth control – OD sample)/OD growth control] × 100.[14,17]

Determination of the minimum fungicidal concentration (MFC)

It is an antimicrobial concentration that can produce 99.9% concentration from the initial concentration. The ethyl acetate fraction of *M. pendens* above the MIC concentration was added to sterile Mueller Hinton agar medium and spread. Then the plate was incubated for 24 h at 37◦C. The lowest concentration that had no visible growth was considered as the MFC. Nystatin was used as a positive control.[18]

Phytochemical screening

The ethyl acetate and hexane fractions of *M. pendens* was screened for the presence of phytochemical compounds, such as flavonoids, tannins, saponins, terpenoids, and phenolics, in accordance with the procedure.[13] Phytochemical reagent was added to the ethyl acetate and hexane fractions. The qualitative results are shown as positive (+) for the presence and

negative (-) for the absence of phytochemicals.[9] This research was an experimental in-vitro study to determine the effect of ethyl acetate and n-hexane fractions of *M. pendens* on the growth of *C. albicans* ATCC 10231 by the calculation of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). This research had ethical clearance no 415/UN6. C1.3.2/KEPK/PN/2015.

RESULTS

The inhibitory test results of ethyl acetate fraction on *C. albicans* showed inhibition diameters at concentrations of 10.000, 5.000, and 2.500 µg/ml. The ethyl acetate fraction had an inhibition diameter of 9.58 mm, 9.08 mm, 9.08 mm. The results of this test indicated that the ethyl acetate fraction had an antifungal effect on *C. albicans*.

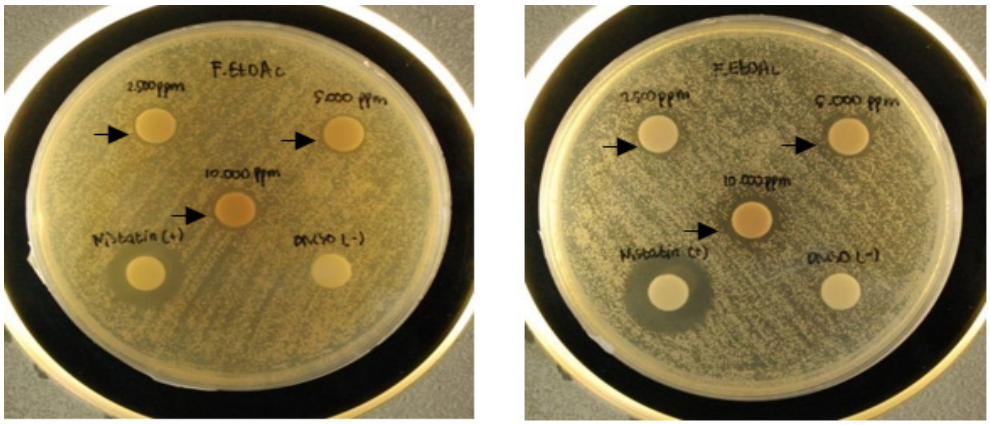

Figure 1. Inhibition zone of ethyl acetate fraction M. pendens against C. albicans

Table 1. The inhibitory test results of M. pendens ethyl acetate fraction on C. albicans

No	Sample concentration (µg/ml)	Diameter inhibition (mm)		Mean	Standard Deviation	
1	Ethyl acetate fraction *M. pendens* 10.000	9.45	9.7	9.58	9.58	Inhibition
2	Ethyl acetate fraction *M. pendens* 5.000	8.75	9.4	9.08	9.08	Inhibition
3	Ethyl acetate fraction *M. pendens* 2.500	8.75	9.4	9.08	9.08	Inhibition
4	Control positive: Nystatin 50	16.9	17.45	17.18	17.18	Sensitive
5	Control negative: DMSO	-	-	-	-	No Inhibition

Note: The inhibitory test was carried out in duplicate, paper disc diameter: 8 mm

Determining the result of inhibitory test was based on method for antifungal disk diffusion susceptibility testing of yeast; approved guideline CLSI M44-A2.[15] Criteria for clear zone, inhibition >20 mm very strong, 10-20 mm strong, 5-10 mm medium, <5 mm weak.[16] Ethyl acetate fraction had zona inhibition 9,08 mm and 9,58 mm that it classified according to medium criteria. The MIC of the ethyl acetate fraction of *M. pendens* was observed by reading optical density with ELISA reader. The percentage of inhibition increased with increasing concentration in table 2. The results of the calculation of the percentage of inhibition of *C. albicans* were obtained at the lowest concentration that can inhibit fungal growth, namely ethyl acetate fraction at a concentration of 625 µg/ml.

Table 2. Percentage of C. albicans inhibition in ethyl acetate fraction of M. pendens

Concentration ethyl acetate fraction (µg/ml)	% Inhibition *C. albicans*
5000	40.5
2500	43.0
1250	125.0
625	52.9
312	-2.3
156	-3.7
78	-30.3
39	-8.5
19	-4.0
10	-6.4
5	-6.4
2	-5.3

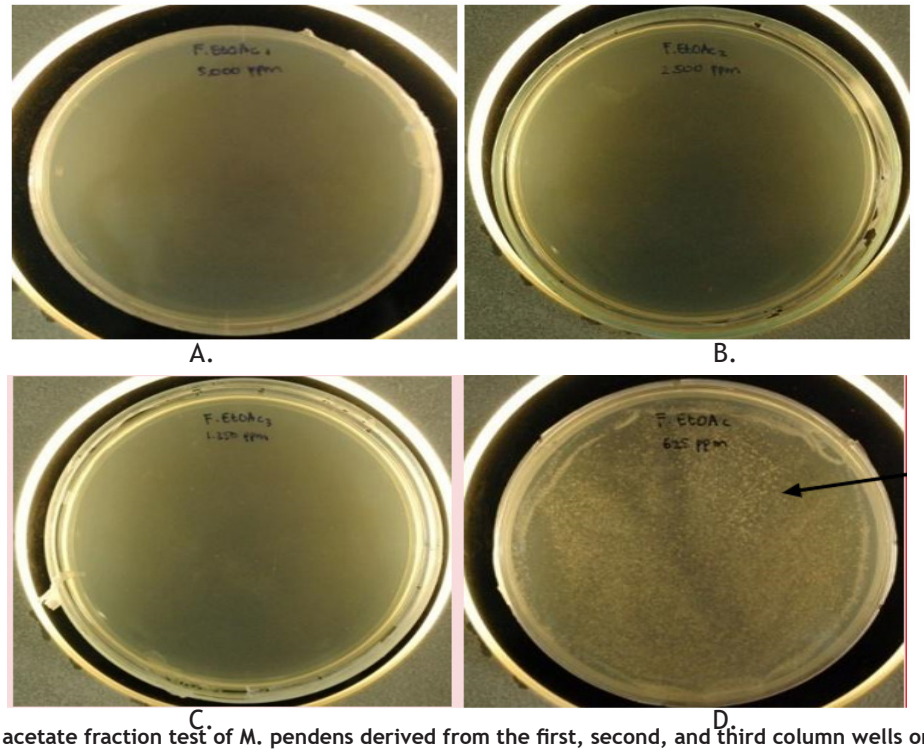

Figure 2. Ethyl acetate fraction test of M. pendens derived from the first, second, and third column wells on the agar plate a) no yeast growth on concentration of 5,000 µg/ml, (b) no yeast growth on concentration of 2,500 µg/ml, c) no yeast growth on concentration of 1,250 µg/ml, d) yeast growth on concentration of 625 µg/ml

Table 3. Phytochemical test of ethyl acetate and n-hexane fractions of M. pendens

	Secondary metabolites	Test method	Ethyl acetate
1	Phenol	$FeCl_3$ 5% reagent	+
2	Flavonoid	a. HCl + Mg reagent	+
		b. H_2SO_4 2N reagent	+
		c. NaOH 10% reagent	+
3	Terpenoid	Lieberman-Burchard reagent	+
4	Saponin	HCl + H_2O reagent	-
5	Tannin	$FeCl_3$ 1% reagent	+

The concentrations of ethyl acetate fraction (625 µg/ml, 1250 µg/ml, 2500 µg/ml, 5000 µg/ml) could inhibit *C. albicans* growth with inhibition percentage 40,5%, 43%, 125%, and 52,9% (table 2). The concentrations of ethyl acetate fraction (2,5 µg/ml - 312,5 µg/ml) had percentage inhibition negative thus there was not inhibition *C. albicans* growth. The MIC value of ethyl acetate fraction was obtained from the lowest concentration that could inhibit candida growth, namely at concentration of 625 µg/ml. Figure 2 showed the agar plate at a concentration of 5.000 µg/ml, 2.500 µg/ml, and 1.250 µg/ml showed no growth of *C. albicans*. The concentration of 625 µg/ml began to show *C. albicans* growth so that MFC ethyl acetate fraction was determined at a concentration of 1.250 µg/ml.

The phytochemical screening of ethyl acetate fraction of M. pendens expressed the presence of some bioactive compounds, such as phenol, flavonoid, terpenoid, and tannin. However, ethyl acetate fraction of M. pendes did not contain saponin.

DISCUSSION

In this study, the concentration of ethyl acetate fraction 2.500, 5.000, 10.000 µg/ml showed an average 9,08-9,58 mm inhibition zone, which means that according to the method of David and Stout, the average diameter of 5-10 mm is categorized as medium. The antifungal ability of ethyl acetate fraction of *M. pendens* was still below nystatin 17,18 mm inhibition zone with strong

antifungal effect. Therefore, it can be developed as the prevention agent of oral candidiasis.[18] The MIC (625 µg/ml) and MFC (1.250 µg/ml) values of ethyl acetate fraction of M. pendens against C. albicans were lower than MIC (1.250 µg/ml) and MFC (2.500 µg/ml) water fraction of M. pendens. This is due to their different content and number of compounds.

Phytochemical test results in this study were ethyl acetate fraction contains phenol, flavonoid, terpenoid, and tannin, whereas water fraction contained phenol, flavonoid, terpenoid, tannin, and saponin. Ethyl acetate fraction of M. pendens can kill and inhibit *C. albicans* which is consistent with previous study was reported by Balafif FF et al. that water fraction of M. pendens had antifungal properties that can kill and inhibit growth of *C. albicans*.[12]

The compound of ethyl acetate fraction was phenol, flavonoid, terpenoid, and tannin. The study Soeksmanto et al obtained that the ethyl acetate fraction contained flavonoids and tannin.[9] The study of Soraya et al tested extract M. pendens. It contained flavonoids, tannins, saponins, alkaloids.[20] The difference in compound content obtained between previous studies and the results of this study might be influenced by differences in extraction methods, solvents usage, and the sensitivity of the fractionation process. Methanol solvent can attract most bioactivity compounds.

Solvent polarity influences in increasing bioactivity compound solubility.[19] Phenol compounds through hydroxy groups that will bind to sulfhydryl groups of fungal proteins so that they can change the shape of cell membrane proteins. The position and number of hydroxyl groups on phenols are related to toxicity to microorganisms. Phenol can denature protein bonds in the cell membrane so that the cell membrane becomes lysis and phenol penetrates into the cell nucleus. The higher the oxidized phenol, the more it will inhibit.[21]

Flavonoid is a type of structure that has antifungal ability against C. albicans. Flavonoid often inhibit fungal growth in various underlying mechanisms by enhancing the disruption of the plasma membrane and mitochondrial dysfunction, inhibiting cell wall formation, cell division, protein synthesis, and the efflux-mediated pumping system. Mechanism of action of flavonoid can damage the function cell membrane and cell wall.[22,23,24] Hydrophobic nature of the terpenoid causes it to enter the lipid membrane. Terpenoid can pass through fungal cell walls. The position is around the lipid bilayer fatty acid chains. Terpenoid interferes with the formation of lipids and changes the structure of the cell membrane. Lipophilic compounds penetrate into cells and interfere with ergosterol biosynthesis.[24] Antifungal effect of tannin can react to the cell wall and penetrate cell membrane.

Tannin can hydrolyze ester groups between galat acids that affect biosynthesis cell wall and cell membrane. The leaking of the cell wall permeability or some changed cell membrane results loss of cytoplasm.[25] The active compounds (phenol, flavonoid, terpenoid, and tannin) from ethyl acetate fraction of M. pendens have ability damage cell membranes and cell walls of *C. albicans*.

CONCLUSION

The ethyl acetate fraction of M. pendens can inhibit the growth of C. albicans. M. pendens can be developed as the prevention agent of oral candidiasis.

ACKNOWLEDGEMENTS

Our immense appreciation is for all colleagues in the Faculty of Dentistry and Faculty of Medicine, Universitas Padjadjaran. This research is part of the Academic Leadership Grant Universitas Padjadjaran.

REFERENCES

1. JW Millsop, N Fazel. Oral candidiasis. Clindermatol J. 2016;34:487-494. DOI: 10.1016/j.clindermatol.2016.02.022
2. Agrawal A, Singh A, Verma R, Murari A. Oral candidiasis: An overview. Journal of Oral and Maxillofacial Pathology. 2014;18(4):81. DOI: 10.4103/0973-029X.141325
3. Ying YL, Lin LM, Lynn MH, Ibrahim N, Abdullah AAA. Presence of pathogenic candida; hyphae in patients with palatal coverafe aplliance/ protheses. Arch Orofac Sci. 2019;14:30-39.

4. Martins N, Ferreira ICFR, Barros L, Silva S, Henriques M. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. Mycopathologia. 2014;177(5-6):223-240. DOI: 10.1007/s11046-014-9749-1
5. Lyu X, Zhao C, Hua H, Yan Z. Efficacy of nystatin for the treatment of oral candidiasis: a systematic review and meta-analysis. Drug Des Devel Ther. 2016:1161. DOI: 10.2147/DDDT.S100795
6. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. 2012.
7. Samadi FM, Suhail S, Sonam M, et al. Antifungal efficacy of herbs. J Oral Biol Craniofac Res. 2019;9(1):28-32. DOI: 10.1016/j.jobcr.2018.06.002
8. 8. Kurnia D, Sumiarsa D, Dharsono HDA, Satari MH. Bioactive Compounds Isolated from Indonesian Epiphytic Plant of Sarang Semut and Their Antibacterial Activity against Pathogenic Oral Bacteria. Nat Prod Commun. 2017;12(8):1934578X1701200. DOI: 10.1177/1934578X1701200814
9. Soeksmanto A, Subroto MA, Wijaya H, Simanjunta P. Anticancer Activity Test for Extracts of Sarang Semut Plant (Myrmecodya pendens) to HeLa and MCM-B2 Cells. Pakistan Journal of Biological Sciences. 2010;13(3):148-151. DOI: 10.3923/pjbs.2010.148.151
10. Sudiono J, Oka C, Trisfilha P. The Scientific Base of Myrmecodia pendans as Herbal Remedies. Br J Med Med Res. 2015;8(3):230-237. DOI: 10.9734/BJMMR/2015/17465
11. Suraini. Uji daya hambat infusum sarang semut (Myrmecodia pendens) yang disimpan menggunakan wadah plastik dan kaca terhadap pertumbuhan Candida albicans. Menara Ilmu. 2017;11(1):205-212.
12. Balafif FF, Satari MH, Dhianawaty D. Aktivitas Antijamur Fraksi Air Sarang Semut Myrmecodia Pendens Pada Candida Albicans ATCC 10231. Majalah Kedokteran Bandung. 2017;49(1):28-34. DOI: 10.15395/mkb.v49n1.984
13. Harbone. JB Phytochemical Methods: A Guide to Modern Techniques of Plants Analysis. Chapman and Hall; 2012.
14. CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. CLSI Standard M.; 2017.
15. Mahon, Connie R; Lehman DC MG. Textbook of Diagnostic Microbiology. Fifth. Saunders Elsevier. 2015.
16. Nikolic M, Vasic S, Djurdjevic J, Stefanovic O, Comic L. Antibacterial and anti-biofilm activity of ginger (Zingiber officinale (Roscoe)) ethanolic extract. Kragujevac Journal of Science. 2014;(36):129-136. DOI: 10.5937/KgJSci1436129N
17. CLSI. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeast. 3rd ed. Clinical Laboratory Standards Institute; 2018.
18. Nasution M, Yendriwati, Hasibuan LGA. Anti-fungal effects of ambon banana skin extract (Musa paradisiaca L var. sapientum) on candida albicans ATCC 10231 (in vitro). JBio Innov. 2014;11(1):197-206.
19. Soraya C, Dharsono HDA, Aripin D, Satari MH, Kurnia D, Hilmanto D. Effects of sarang semut (Myrmecodia Pendens Merr. & Perry) extracts on Enterococcus faecalis sensitivity. Dental Journal (Majalah Kedokteran Gigi). 2016;49(4):175. DOI: 10.20473/j.djmkg.v49.i4.p175-180
20. Thouri A, Chahdoura H, el Arem A, Omri Hichri A, ben Hassin R, Achour L. Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). BMC Complement Altern Med. 2017;17(1):248. DOI: 10.1186/s12906-017-1751-y
21. Vikrant P, Jaitly, Kunchiraman. Plants with anti-Candida activity and their mechanism of action: a review. Environ Res Dev J. 2015;9:1189-1196.
22. Arif T, Bhosale JD, Kumar N, et al. Natural products – antifungal agents derived from plants. J Asian Nat Prod Res. Published online July 2011:283-311. DOI: 10.1080/10286020902942350.
23. Aboody MS, Mickymaray S. Anti-Fungal Efficacy and Mechanisms of Flavonoids. Antibiotics. 2020;9(2):45. DOI: 10.3390/antibiotics9020045
24. Edziri H, Mastouri M, Mahjoub MA, Mighri Z, Mahjoub A, Verschaeve L. Antibacterial, Antifungal and Cytotoxic Activities of Two Flavonoids from Retama raetam Flowers. Molecules. 2012;17(6):7284-7293. DOI:

10.3390/molecules17067284

25. Mailoa MN, Mahendradatta M, Laga A, Djide N. Antimicrobial activities of tannins extract from guava leaves (Psidium guajava L) on pathogens microbial. Inter J Scie Tech Res. 2014;3:236-241.