RESEARCH NOTE

Preliminary study on the inhibitory effect of seaweed *Gracilaria verrucosa* extract on biofilm formation of *Candida albicans* cultured from the saliva of a smoker [version 1; peer review: 3 approved with reservations]

Zaki Mubarak¹ , Adintya Humaira¹, Basri A. Gani¹, Zainal A. Muchlisin²

¹Faculty of Dentistry, Syiah Kuala University, Banda Aceh, 23111, Indonesia

²Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh, 23111, Indonesia

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Abstract

Background: *Candida albicans* is an opportunistic fungus that infects the oral cavity. Increases in colony numbers of *C. albicans* can be caused by multiple factors, such as smoking, a weakened immune system, taking antibiotics and with immune-compromised individuals. Smoking can increase the virulence factor of *C. albicans* and make it stronger. One of the virulence factors of *C. albicans* is the biofilm it forms. The *C. albicans* biofilm makes it more tolerant to extracts of the seaweed *Gracilaria verrucosa*, which has antifungal activity. The objective of the study was to examine the ability of the *G. verrucosa* extracts to inhibit the formation of biofilm by *C. albicans* obtained from the saliva of smoker.

Methods: A total of six concentrations of *G. verrucosa* (6.25, 12.5, 25, 50, 75 and 100%) were tested in this study. The positive control was fluconazole 0.31 µg/ml. *C. albicans* was taken from the saliva of one smoker in Faculty of Dentistry, Syiah Kuala University. The total amount of biofilm was assessed using an ELISA reader. The data were subjected to Kruskal-Wallis test at a significance limit of $p < 0.05$.

Results: The seaweed extract has three bio-active compounds: steroids, terpenoid, and tannins. The results showed that the inhibitory activity of seaweed on *C. albicans* biofilm formation increases as its concentration increases. The highest effectiveness was recorded at a seaweed concentration of 100% at 48 h of exposure.

Conclusions: The optimal inhibition of the *C. albicans* biofilm formation was recorded at the concentration of 100% *G. verrucosa* after 48 hours of exposure.

Open Peer Review

Approval Status

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version 3			
(revision)			
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version 2			
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31 May 2018	 view	 view	 view

1. **Heni Susilowati** , Gadjah Mada University, Yogyakarta, Indonesia
2. **Shahida Mohd-Said** , Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
3. **Cristiane Y. Koga-Ito** , São Paulo State University - UNESP, São José dos Campos, Brazil

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

Candida albicans, oral candidiasis, seaweed *Gracilaria verrucosa*

Corresponding author: Zaki Mubarak (zakimubarak54@yahoo.com)

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Introduction

Smoking is a common problem in most developing countries, including Indonesia. Based on a survey by The Tobacco Atlas in 2015, Indonesia has the highest number of smokers in Asia, with 66% of men in Indonesia being active smokers¹. Smoking can lead to addiction owing to the nicotine contents, and harm due to the presence of toxic compounds such as CO, ammonia and tar contents in tobacco¹. Besides causing addiction, substances in cigarettes can also cause various diseases, such as oral candidiasis. Oral candidiasis is caused by the infection of the fungus *Candida albicans*². This fungus is part of the normal flora of the human mouth, but it can become pathogenic in certain conditions, for example, due to nicotine exposure³.

Infection with *C. albicans* will increase the formation of a biofilm of the fungus³. The biofilm is an extracellular matrix consisting of *C. albicans* colonies⁴. The size of the biofilm increases when exposed to substances in cigarette smoke, as the cigarette has content that can initiate growth and nourish *C. albicans*^{5,6}.

Currently, fluconazole and nystatin are the most effective drugs for treating oral candidiasis. Unfortunately, these drugs have side effects; for example the prolonged use of fluconazole leads to resistance⁷, while high dosages of nystatin give gastrointestinal discomfort and increase plaque formation⁸. Therefore, plant-derived antifungals may be a viable oral treatment option for candidiasis. One of these potential plants is seaweed *Gracilaria verrucosa*. This seaweed contains several bioactive compounds, including alkaloids, flavonoids, phenolics, saponins, steroids and terpenoids⁹. Aceh Province, Indonesia, has large *G. verrucosa* resources, although so far this aquatic plant has not been commonly used for medicinal purposes. Hence, the objective of the present study was to examine the ability of seaweed extract to inhibit the growth of *C. albicans* obtained from smoker saliva, as indicated by biofilm formation.

Methods

Time and site

The study was conducted in August 2017 at The Laboratory of Microbiology, Veterinary Faculty, Syiah Kuala University. *C. albicans* was extracted from the saliva of one volunteer active smoker volunteer in Faculty of Dentistry Medicine, Syiah Kuala University. The volunteer was asked directly and accepted, giving written informed consent. The inclusion criteria of the volunteer was an active smoker that smoke at least 20 cigarettes per day. The saliva was collected once the volunteer finished smoking, the *G. verrucosa* seaweed was collected from a farmer in Pulo Aceh, Aceh Province. The subject provided written informed consent to participate in this study. Ethical clearance (No. 1741/UN11.1.21/TU/2017) was obtained from Faculty of Dentistry, Syiah Kuala University, Banda Aceh, Indonesia.

Seaweed extraction

Extraction was performed based on the Maserati method¹⁰. A total of 3 kg seaweed was washed with tap water then rewashed using distilled water. The seaweed sample was dried at room temperature 25°C for 24 h, avoiding direct sunlight. The wet

seaweed was chopped into small-sized pieces (2 mm), then soaked in 96% ethanol, as a solvent. After 24 h the sample was filtered using Whatman filter paper No. 42 and the resulting residue was soaked again in 96% ethanol. This procedure was repeated until the solvent color which added to sample was not changing the color or limpid. All the filtrate collected in all of the procedures was then evaporated using a vacuum rotary evaporator (Laborta 4003 control, Heildolph) for 15 min at 60°C. The extract was taken and stored in a refrigerator at 4°C.

Saliva collection

The saliva was collected from a volunteer active smoker in Faculty of Dentistry Medicine, Syiah Kuala University. Saliva was collected by spitting into a glass jar (15 ml), then 1 ml PBS (0.01 M, pH 7.2) was added to the jar. The jar was centrifuged at 10,000 rpm for 10 min, after which the precipitate was taken and incubated in CHROMagar *Candida* medium for 2 days to allow for colony development. If the colour of a colony was green, this indicated that the colony was *C. albicans*.

C. albicans suspension preparation

Following culturing of *C. albicans* in CHROMagar *Candida* medium, one colony of cultured *C. albicans* was mixed with 5 ml peptone in a tube then incubated at 37°C for 24 h. After 24 h, the turbidity of media was compared to a 0.5 McFarland solution standard, equivalent to 1.5×10^8 CFU/ml.

Phytochemical tests

Flavonoid test. A total of 5 ml seaweed extract were mixed with 0.5 cm Mg band and two drops of HCl then heated by passing over a Bunsen flame. The coloration to red or purple after heating indicated the presence of flavonoids¹¹.

Alkaloid tests. A total of 5 ml seaweed extract and 8 ml HCl were mixed to homogeneity then filtered. The filtrate was then subjected to Mayer, Wagner and Dragendorff tests for alkaloids to ensure detection of any alkaloids, based on those described by Vimalkumar *et al.*¹¹. For the Mayer test, approximately 2 ml filtrate was mixed with 5 g potassium mercuric iodide. The formation of white or pale precipitates indicates the presence of alkaloids. For the Wagner test, a total of 2 ml filtrate was mixed with 2 ml Wagner reagent. The formation of brown or reddish-brown precipitates indicates the presence of alkaloids. For the Dragendorff test, 2 ml of filtrate was mixed homogeneously with bismuth potassium iodide solution, the red precipitates indicate the presence of alkaloid.

Tannin/phenolic test. Two drops of 1% FeCl₃ was added to 1 ml seaweed extract. The change in the color to a blackish green indicates the presence of tannin/phenolic content¹².

Saponin test. A total of 1 ml seaweed extract was mixed with distilled water to 20 ml then shaken vertically for 15 s. Persistent foaming is indicative of saponin content.

Steroid test. Approximately 2 ml seaweed extract was diluted in 2 ml CHCl₃, a few drops of H₂S and 1 ml of CH₃COOH. The formation of green or blue precipitates indicates the presence of steroid¹¹.

Terpenoid test. A total of 5 ml seaweed extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated H_2SO_4 . A layer of the reddish brown coloration was formed at the interface thus indicating a positive result for the presence of terpenoids¹³.

Biofilm examination

A total of 100 μ l casein-peptone lecithin polysorbate broth (Merck-1117230500) was prepared in each well of a 96-well plates for 5 min then the peptone was removed from the wells. A total of 50 μ l cultured *C. albicans*, which diluted to a 0.5 McFarland standard turbidity, were added into 96-well plates and left in wells for 5 min. Next, the seaweed extracts were added at decreasing concentrations test (100, 75, 50, 25, 12.5 and 6.25%), with fluconazole 0.31 μ g/ml as a control. The plates were incubated for 24, 48 or 72 h at 37°C, then approximately 200 μ l of 0.1% violet crystal were added into the plates and incubated for 15 min at room temperature.

After 15 min, each well was washed three times with 200 μ l PBS. The crystal violet in each well was then dissolved

in 100 μ l 96% ethanol for 2 min. The biofilm formation was analyzed using an ELISA reader at 620 nm wavelength^{14,15}.

Data analysis

The data were subjected to Kruskal-Wallis test using SPSS software v20.0.

Results

The results of phytochemical tests, showed that seaweed *G. verrucosa* extract had the positive reaction to a steroid, terpenoid, and tannin indicates these substances are present in the seaweed (Table 1).

In general, the inhibitory effect was increased as seaweed concentration increased. Results of Kruskal-Wallis analysis ($P < 0.05$) showed that seaweed extract significantly inhibited formation of the biofilm of *C. albicans*. However, a higher optical density was recorded for fluconazole (control), followed by 100% seaweed extracts in all exposure times; there were no significant differences between these treatments. The results showed that the best inhibition effect was recorded with fluconazole followed by 100% seaweed extract 48 h after exposure (Figure 1).

Table 1. Phytochemical analysis of seaweed extract *Gracilaria verrucosa*.

Substance	Reagent	Result	Indication
Alkaloid	Mayer	-	White deposit
	Wagner	-	Brown deposit
	Dragendroff	-	Red deposit
Steroid	Uji Lieberman-Burchard	+	Green or blue colors
Terpenoid	Uji Lieberman-Burchard	+	Red or purple colors
Saponin	Shuffling method	-	Stable foams
Flavonoid	0.5 Mg and HCl	-	Red or purple colors
Tannin/Phenolic	$MgCl_3$	+	Dark green

Figure 1. The formation of *Candida albicans* biofilm exposed to *Gracilaria verrucosa* seaweed extract, based on different exposed time and extract concentration.

Dataset 1. The raw data of the Triplo anti-Biofilm seaweed to *C. albicans* for 24, 48 and 72 h at a wavelength 620 nm

<http://dx.doi.org/10.5256/f1000research.14879.d204270>¹⁶

Discussion

The study showed that 100% seaweed extract is promising for inhibiting the growth of *C. albicans*, indicating that it has the potential to be used as an anti-fungus *C. albicans* to treat oral candidiasis in smokers. *C. albicans* is a normal micro-organism in the human mouth; however, this fungus can be pathogenic in certain circumstances³, such as in the mouth of smokers². Smoking can increase the protein levels of HWP1, EAP1 and SAP2 in *C. albicans*. Higher levels of these proteins increase the virulence of *C. albicans*. This can then increase biofilm formation and cause oral candidiasis⁶. In addition, smoking can also cause a decrease in immune function, making individuals more susceptible to oral candidiasis^{4,6}.

The results showed that treatment with 100% seaweed extract can inhibit the formation of *C. albicans* biofilm to an almost equivalent degree as the fluconazole (control). This activity is presumably caused by the bioactive compounds in the extract of seaweed, such as the steroids, terpenoids, and tannins that were detected in this study. According to Sampaio *et al.*¹⁷, the anti-fungal activity of a substance strongly depends on the composition of its bio-active compounds; these bio-active compounds have the potential to cause destruction to the biofilm and affect the viability of *C. albicans*; for example, steroids can kill *C. albicans* through their lipophilic properties, interfering with the formation of fungal spores and mycelium¹⁸. This activity weakens *C. albicans*, inhibiting the formation of the biofilm. The activity of the steroids requires oligosaccharides that are also present in the seaweed content to function optimally¹⁹.

Terpenoids are derivatives of saponins. Terpenoids act as an anti-fungals by damaging the organelles of the fungi and inhibiting the secretion of enzymes, leading to inhibition of the growth of *C. albicans* fungal cells. Terpenoids can also damage the morphology of *C. albicans*²⁰. Tannins may inhibit chitin synthesis

in *C. albicans* cell walls; as a result, there is no protection of the *C. albicans* cell membrane and can cause inhibit cellular metabolism. In addition, tannin can inhibit ergosteron activity of *Candida albicans*²¹.

The effectiveness of the extracts in inhibiting fungi is influenced by at least three factors, namely the concentration, exposure time, and contact surface media²². The present study showed that the inhibitory effect of seaweed extract increased as seaweed extract concentration increased, with the best effect recorded at 48 h of exposure, this is probably because the farnesol works effectively after 48-72 h of exposure. Farnesol is a quorum-sensing molecule that has the potency to inhibit *C. albicans* growth²³.

Further studies should be conducted to extract the individual bioactive compounds in seaweed then test their action on *C. albicans* at different dosages. The purpose of these further studies will be to assess which bioactive compound, and at which dosages, are playing a vital role in inhibiting the growth of *C. Albicans*.

Conclusion

Gracilaria verrucosa seaweed extract inhibited the growth of the biofilm of *C. albicans* isolated from the saliva of a smoker, with the inhibitory effect increasing with the concentration, up to an optimal concentration of 100% at 48 h of exposure.

Data availability

Dataset 1. The raw data of the Triplo anti-Biofilm seaweed to *C. albicans* for 24, 48 and 72 h at a wavelength 620 nm. DOI: [10.5256/f1000research.14879.d204270](https://doi.org/10.5256/f1000research.14879.d204270)¹⁶.

Competing interests

No competing interests were disclosed.

Grant information

The authors declare that no funding was involved in supporting this work.

References

- Lian TY, Dorotheo U: **The Tobacco Control Atlas**. Seutawan Co. 2016; 3. [Reference Source](#)
- Mayer FL, Wilson D, Hube B: ***Candida albicans* pathogenicity mechanisms**. *Virulence*. 2013; 4(2): 119–128. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Adel E, Khadijeh PS, Mohammad M: **Oral Cavity Candidiasis as a Complication of Fungal Diseases in Diabetic Patients in South-East of Iran**. *IJAS*. 2016; 14(4): 1134–1138. [Reference Source](#)
- Little JW, Falace D, Miller C: **Dental Management of The Medically Compromised Patient**. Mosby: Elsevier; 2013. [Reference Source](#)
- Keten HS, Keten D, Ucer H, *et al.*: **Prevalence of oral candida carriage and candida species among cigarette and maras powder users**. *Int J Clin Exp Med*. 2015; 8(6): 9847–54. [PubMed Abstract](#) | [Free Full Text](#)
- Semlali A, Killer K, Alanazi H, *et al.*: **Cigarette Smoke Condensate Increases *C. albicans* Adhesion, Growth, Biofilm Formation, and EAP1, HWP1 and SAP2 Gene Expression**. *BMC Microbiol*. 2014; 14: 61. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Peron IH, Lima FR, Lopes AF, *et al.*: **Resistance Surveillance in *Candida albicans*: A Five-Year Antifungal Susceptibility Evaluation in a Brazilian University Hospital**. *PLoS One*. 2014; 11(7): e0158126. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

8. Lyu X, Zhao C, Yan MZ, *et al.*: **Efficacy of Nystatin for The Treatment of Oral Candidiasis: a Systematic Review and Meta-Analysis.** *Drug Des Devel Ther.* 2016; **10**: 1161–1171.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Sahat JH: **Warta Ekspor.** September ed. Jakarta: DGNE; 2013; 3.
10. Prima MI: **Uji Aktivitas Antibakteri Ekstrak Metanol Ganggang Merah *Gracilaria verrucosa* terhadap Beberapa Bakteri Patogen Gram Positif dan Gram Negatif.** Published Only In Database. 2012.
[Reference Source](#)
11. Vimalkumar CS, Hosagaudar VB, Suja SR, *et al.*: **Comparative Preliminary Phytochemical Analysis Of Ethanolic Extracts of Leaves of *Olea dioica* Roxb., Infected with The Rust Fungus *Zaghouania oleae* (E.J.Butler) Cummins and Non-infected Plants.** *J Pharmacogn Phytochem.* 2014; **3**(4): 69–72.
[Reference Source](#)
12. Hariyanto Ih, Inarah F, Suci PR, *et al.*: **Skrining Fitokimia dan Analisis Kromatografi Lapis Tipis dari Ekstrak Ethanol Herba Pacar Air (*Impatiens balsamina* Linn.).** Published Only In Database. 2014.
[Reference Source](#)
13. Khan MA, Qureshi AR, Ullah F, *et al.*: **Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings.** *JMPR.* 2011; **5**(25): 6017–7.
[Reference Source](#)
14. Metzler A: **Developing a Crystal Violet Assay to Quantify Biofilm Production Capabilities of *Staphylococcus aureus*.** Published Only In Database. 2016.
[Reference Source](#)
15. Roberts SK, Wei GX, Wu CD: **Evaluating biofilm growth of two oral pathogens.** *Lett Appl Microbiol.* 2002; **35**(6): 552–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
16. Mubarak Z, Humaira A, Gani BA, *et al.*: **Dataset 1 in: Preliminary study on the inhibitory effect of seaweed *Gracilaria verrucosa* extract on biofilm formation of *Candida albicans* cultured from the saliva of a smoker.** *F1000Research.* 2018.
[Data Source](#)
17. Sampaio BL, Edrada-Ebel R, Da Costal FB: **Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: a model for environmental metabolomics of plants.** *Sci Rep.* 2016; **6**: 29265.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Subsiha S, Subramoniam A: **Antifungal Activities of Steroid From *Pallavicinia lyellii*, a liverwort.** *Indian J Pharmacol.* 2005; **37**(5): 304–308.
[Publisher Full Text](#)
19. Cammarata A, Upadhyay SK, Jursic BS, *et al.*: **Antifungal activity of 2 α ,3 β -functionalized steroids stereoselectively increases with the addition of oligosaccharides.** *Bioorg Med Chem Lett.* 2011; **21**(24): 7379–7386.
[PubMed Abstract](#) | [Publisher Full Text](#)
20. Martínez A, Rojas N, García L, *et al.*: **In vitro activity of terpenes against *Candida albicans* and ultrastructural alterations.** *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2014; **118**(5): 553–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
21. Hastuti SU, Ummah PIY, Khasanah NH: **Antifungal Activity of *Piper aduncum* and *Peperomia pellucida* Leaf Ethanol Extract Against *Candida albicans*.** *AIP Conf Proc.* 2017; **1844**: 020006.
[Publisher Full Text](#)
22. Kenakin PT: **A Pharmacology Primer: Theory, Application, and Methods.** 2nd edition. USA: Elsevier. 2006; 36–38.
[Publisher Full Text](#)
23. Alem AM, Oteef MD, Flowers TH, *et al.*: **Production of tyrosol by *Candida albicans* biofilms and its role in quorum sensing and biofilm development.** *Eukaryot Cell.* 2006; **5**(10): 1770–1779.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Version 1

Reviewer Report 27 June 2018

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Cristiane Y. Koga-Ito

Oral Biopathology Graduate Program, Institute of Science and Technology, São Paulo State University - UNESP, São José dos Campos, Brazil

The manuscript by Mubarak et al. aimed to evaluate the effect of *G. verrucosa* extract on *Candida albicans* biofilm formation. The rationale of the study should be clearer. Also, there is lack of essential information throughout the text. The English should be revised.

Specific comments

Introduction

The period "Smoking is a common problem in most developing countries, including Indonesia.". A reference should be added. Also, smoking is not a problem only or mainly in developing countries. Please, consider revising.

The authors stated that "This fungus is part of the normal flora of the human mouth, but it can become pathogenic in certain conditions, for example, due to nicotine exposure.". The exposure to nicotine has been correlated with increasing in *C. albicans* virulence factor expression. The predisposing conditions for candidiasis are much more related to immunologic state of the host and imbalance in microflora. Please, consider revising.

Revise the period "Infection with *C. albicans* will increase the formation of a biofilm of the fungus", it is confusing.

Revise the period "The biofilm is an extracellular matrix consisting of *C. albicans* colonies", it is confusing.

The authors stated that "high dosages of nystatin give gastrointestinal discomfort and increase plaque formation". What do authors mean by "increase plaque formation"?

The rationale of the study is not clear. Why did the authors select *G. verrucosa*? Is this plant commonly used? Why did the authors decide to use saliva from a smoker individual?

Methods

Revise the period "*C. albicans* was extracted from the saliva", *C. albicans* was isolated from saliva. The authors reported that "*G. verrucosa* seaweed was collected from a farmer in Pulo Aceh, Aceh

Province.". More information on the plant, the exact location it was collected from, the period of the year, identification procedure (how and who did the identification?), registration in herbarium, number of voucher should be included in the text.

Please, revise the period "Saliva was collected by spitting into a glass jar (15 ml), then 1 ml PBS (0.01 M, pH 7.2) was added to the jar." Why did 1 ml of PBS added to the saliva? What was the final volume of saliva collected? Was saliva stimulated?

The authors stated that "If the colour of a colony was green, this indicated that the colony was *C. albicans*.". However, the color of the colony in CHROMagar is only a presumptive test and phenotypic or genotypic definitive identification should be done.

The inclusion of a reference strain is highly needed.

Include the number of experiments/replicates performed.

The inclusion of more clinical isolates from non-smokers patients is needed.

The methodology of activity of extract on biofilm formation is not clear. Why and how peptone was removed from the wells? Why did the fungal suspension left in the wells for 5 min? How 100% extract concentration was obtained in the well (you already has the broth inside the well)? Why the concentration of 0.31 was chosen for fluconazole?

Figure 1 should be revised. Note that optical density at 620 nm is higher after treatment with 100% extract when compared to the other concentrations.

Discussion and Conclusion sections should be revised after the revision of the aforementioned points.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 19 June 2018

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Shahida Mohd-Said

Periodontology Unit, Centre for Restorative Dentistry, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Overall fair manuscript with sound finding and relevant area of study. Can much be improved with grammar check and essential scientific writing reorganisation especially in Introduction and Discussion section. Inclusion of results for negative control (untreated biofilm) would critically improve Results presentation and appreciation of findings. Lacks relevant information on findings between smoker and non-smoker in the study that may not strongly support the conclusion on the anti-fungal effect of agent on smoker.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Periodontology, natural product drug discovery

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Aug 2018

Zaki Mubarak, Syiah Kuala University, Banda Aceh, Indonesia

Some improvement in grammar, sentences and writing reorganization have been done as a response to reviewer Dr Shahida M. Said. Some details in methods and analysis have also been provided to make better readability and potential reproducibility of the work. As a result the work in this study is much better presented. These improvements can be found in track changes.

As this study is still preliminary comparison between smoker and non-smoker has not been performed yet, but the finding clearly show potential of use of seaweed extract to treat oral candidiasis.

Competing Interests: No competing interests were disclosed.

Reviewer Report 12 June 2018

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Heni Susilowati

Department of Oral Biology, Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia

This preliminary research is interesting enough to be developed but there are some things that need to be reconsidered:

1. ABSTRACT

- Research background that written on the abstract (lines 6 and 7) does not match the purpose of the study. The sentence can be interpreted in contrast to the antifungal potential possessed by *Garcinia verrucosa*. It is dubious to investigate the potency of biofilm inhibition effect if *Candida albicans* more tolerant to *Gracilaria* extract.

2. METHODS

Methods need to explain the following:

- Systemic conditions and state of teeth and oral soft tissue volunteers,
- The predestined determination of the *Gracilaria verrucosa* plant should be mentioned.
- Were the culture washed after the incubation period on treatment?
- How many times an experiment produces a representative result?

3. RESULTS

- Interpretation of the results is confusing; as far as I know the higher the optical density value the more biofilms are formed. The results in Fig. 1 show that 100% and Fluconazole extracts have higher optical densities rather than the lower concentration of extracts, as far as I know this shows that the mass of biofilms formed in the group is higher. Please observe the methods and results of research reported by Sebaa et al 2016.
- The statistical method used was only Kruskal-Wallis, is there a multiple difference analysis? Researchers need to discuss the effect of antibiofilm extract at lower concentrations, because of course 100% extract is not a good recommendation for subsequent experimental use.

References

1. Sebaa S, Hizette N, Boucherit-Otmani Z, Courtois P: Dose-dependent effect of lysozyme upon *Candida albicans* biofilm. *Mol Med Rep*. 2017; **15** (3): 1135-1142 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Aug 2018

Zaki Mubarak, Syiah Kuala University, Banda Aceh, Indonesia

Abstract

Comments from reviewer :

The objective has been clarified

Action done:

We think aims of study still need to be stated

Rearrangement and sentences improvement were done in some sections of abstract

Introduction

Comments from reviewer :

No comments

Action done:

Grammar check, revise and improvement were done to provide more concise and clear sentences/statements

Material and method

Comments from reviewer :

Need clarification on sentence:

"This volunteer has a bad OHIS4" (time and place)

".Then microtiter wells plate wells were washed three times with 200µl of PBS buffer and dried for 15 minutes."(biofilm examination)

"This examination was repeted three times with triplo method"(biofilm examination)

Action done:

Sentences improvement, grammar check and correction had been done throughout this section to provide better readability, clarity and possible reproducibility

Results

Comments from reviewer :

No comments

Action done:

Rewritten this part due to misinterpretations we did in the first previous manuscript about which treatment gave best inhibition effect on *C. albicans*

Discussion

Comments from reviewer :

No specific comments

Action done:

Rewritten has been done to this part in realtion to due to changes in results presentation Sentences improvement, grammar check and reorganization had been done throughout this section to provide better readability and clarity

Conclusion

Comments from reviewer :

No specific comments

Action done:

This part had been rewritten in relation to the revision performed in result and discussion.

Competing Interests: No competing interests were disclosed.

Comments on this article

Version 1

Author Response 05 Aug 2018

Zaki Mubarak, Syiah Kuala University, Banda Aceh, Indonesia

Some revisions and improvements have been done throughout the manuscript as responses to the good comments and suggestions from reviewers. Major revisions performed include grammar check and corrections; sentence improvements; and reorganization and adding appropriate details in each section of the material and methods part. Grammar issues, were concerned by all reviewers, had been addressed. The ways to present results were also changed as response to comment from Indonesia reviewer about the meaning of OD values in presenting the growth of *C. albicans* and how to interpret them. Restructure of result and discussion, therefore, was also performed. These changes became the basis for synthesizing more appropriate conclusion sentences. One reference was also added to support discussion about the importance of extract concentration for resulting in expected medicinal effects, in this study was growth inhibition that reflected by biofilm formation. Since this study is still in preliminary step, no non-smoker participant was participated. As consequence, no comparison could be made between smoker and non-smoker groups. Yet the study was still able to confirm potential of seaweed *Gracia verrucosa* extract as anti candidiasis in smokers. Potential of doing further studies was mentioned in relation to this weakness of our study.

Competing Interests: No competing interests were disclosed.

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