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Poster Session

A-1~A-10: Dental Education
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D-1~D-23: Oral Health and Clinical Treatments

B-3
01-5

The antifungal effect of *Stichopus hermanii* extract to *Candida albicans* in vitro

K. Parisihni[1,3], S. Revianti[1,3] and D. Pringgenies[2]

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BACKGROUND : Sea cucumbers have long been used for food and folk medicine in the communities of Asia. Regarding to the bioactive compound, some species of sea cucumber have been known to have the biomedical properties as antifungal agent. Oral candidiasis is the most common fungal infection in oral cavity caused by *Candida albicans*. An antifungal agent of natural resource will add the great value on the therapy of oral disease. In this preliminary study, golden sea cucumber (*Sticophus hermanii*) was examined its possible antifungal activity towards *Candida albicans* in vitro.

OBJECTIVE : The aim of this study was to examine the antifungal effect of *Stichopus hermanii* extract to the growth of *Candida albicans*.

MATERIAL AND METHOD : The study was an experimental laboratories research with post test only control group design. Three concentration of *Stichopus hermanii* methanolic extract: 20 mg/mL, 40 mg/mL, 80 mg/mL, were tested its antifungal effect against *Candida albicans* by disk diffusion method. The treatment groups were compared to Nystatin oral solution 100.000 IU/ml as positive control and DMSO 1% as negative control. The antifungal effect was examined by measure the diameter of the clear zone around the disk. Data was analyzed by Anova, followed by LSD test.

RESULTS : The result of this study showed the clear zone around the disc of *Stichopus hermanii* extract in all concentrations. It had been proved that antibacterial action of extract *Sthichopus herrmanii* could inhibit the growth *Candida albicans* ($p < 0.05$). The largest diameter of the clear zone around the disc was in the concentration of 80 mg/ mL.

CONCLUSION : *Stichopus hermanii* extract had the antifungal effect against *Candida albicans*. Further in vivo study need to be conducted to explore the potential use of the extract as antifungal agent.

The antifungal effect of Stichopus hermanii extract to Candida albicans in vitro

Kristanti Parisihni[1,2], Syamsulina Revianti[1,2], Delianis Pringgenies[3]

[1] Hang Tuah University, Surabaya, Indonesia, [2]PhD Student of Airlangga University, Surabaya, Indonesia, [3]Diponegoro University, Semarang, Indonesia

BACKGROUND

Indonesian sea possess many species of sea cucumber, *Sticophus hermanii* is the one of the popular ones[1]. Instead of its economical used as meal consume, it was reported in some research that *Sticophus hermanii* as some other species of sea cucumber proved to have some medical properties[2,3].

Sticophus hermanii

Kingdom	: *Invertebrata*
Phylum	: *Echinodermata*
Class	: *Holothuroidea*
Ordo	: *Aspidochirotda*
Family	: *Sticophoidae*
Genus	: *Sticophus*
Species	: *Sticophus hermanii*

Therapeutic properties and medicinal benefits of sea cucumbers can be linked to the presence of a wide array of bioactives especially triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan (GAGs), sulfated polysaccharides, sterols (glycosides and sulfates), phenolics, cerberosides, lectins, peptides, glycoprotein, glycosphingolipids and essential fatty acids. Generally, most species of sea cucumber share the same bioactive compound mentioned above but in different level contain[4,5,6].

Candidiasis is the most common fungal infection in oral cavity, its prevalence raised specially along with the raise prevalence of HIV-AIDS[7,8]. A natural source of antifungal agent could become the novel alternative solution in therapy of oral candidiasis .

Extract of sea cucumber has been known to have: antibacterial and antifungal properties

Sticophus hermanii extract has been proved to have antibacterial effect against Gram positive and negative bacteria : *Escherchia coli, Pseudomonas* sp., *V.vionicica , Staphylococcus aureus, Streptococcus mutans* [6,9,10,11]

Holothuria atra , Holothuria scabra and some holothurians species have antifungal action to *Candida albicans , Aspergillus sp* [11,12,13].

OBJECTIVE :

to examine the antifungal effect of *Stichopus hermanii* extract to the growth of *Candida albicans*

MATERIAL and METHOD

Experimental laboratories research with post test only control group design

Extract Procedure

Samples of *Sticopus hermanii were* collected from Karimunjawa coastal region.

↓

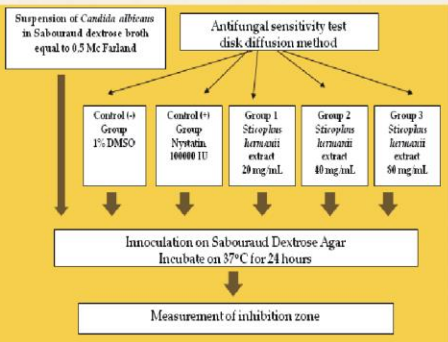
Cleaned, immersed in water for 24 hr, dried in dryer machine, cut in small piece of 3-10 cm.

↓

Extraction process : maceration with methanol solvent (1 : 4) 48 hr. Filtrate separation from solvent → rotary evaporator *40°C*

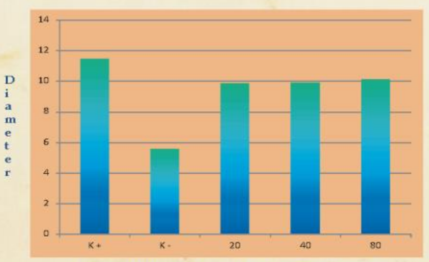
↓

Each xtract then diluted with 1%DMSO into 3 groups concentration of 20 mg/mL, 40 mg/mL, 80 mg/mL

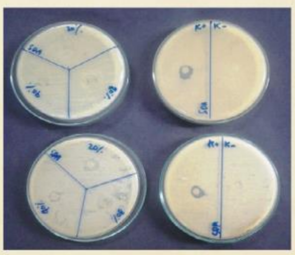


RESULT

The Result of Disc Difussion Method



Concentration of *Sticophus hermanii* extract



RESULT

ANOVA and LSD test : Inhibition Zone Diameter (in mm)

(I) Group	(J) Group	Mean Difference (I-J)	Sig.
K- (Negative Control Group)	K+ (Positive Control Group)	5,90933*	,000*
	Stichopus Hermanii 20mg/ml	1,63933*	,002*
	Stichopus Hermanii 40mg/ml	1,58067*	,003*
	Stichopus Hermanii 80mg/ml	1,34733*	,011*
K+ (Positive Control Group)	Stichopus Hermanii 20mg/ml	-4,27000*	,000*
	Stichopus Hermanii 40mg/ml	-4,32867*	,000*
	Stichopus Hermanii 80mg/ml	-4,56200*	,000*
Stichopus Hermanii 20mg/ml	Stichopus Hermanii 40mg/ml	-,05867	,910
	Stichopus Hermanii 80mg/ml	-,29200	,573
Stichopus Hermanii 40mg/ml	Stichopus Hermanii 80mg/ml	-,23333	,652

ANOVA result showed significant difference between group ($p < 0.05$)

DISCUSSION

Result of antifungal sensitivity test showed inhibition zone in all treatment group and in control positive group, means that extract of *Sticophus hermanii* has antifungal effect to C. *albicans in vitro* The largest diameter of inhibition zone of treatment group was in te concentration of 80 mg/mL but still less than in the control group of *Nystatin* (*p< 0,05*)

Nystatin is a polyene antifungal drug to which many molds and yeasts are sensitive, including Candida spp, used as the positive control for it's the common topical antifungal agent therapy on oral candidiasis. Nystatin exerts its antifungal activity by binding to ergosterol found in fungal cell membranes. Binding to ergosterol causes the formation of pores in the membrane. Potassium and other cellular constituents leak from the pores causing cell death[7,8].

Sea cucumber extract have been known to have the antifungal property, assumed to be related to its content of alcaloid, saponin and triterpen glycoside[3,,12,13,14]

Saponin were identified in the content of sea cucumber extract[2,14]. It is secondary metabolites of glycosidic nature widely distributed in higher plants and marine invertebrates → resulted as the defend mechanism also has the biological properties :

saponin

- Ability to lyse erythrocytes or to foam. It form complexes with cell membrane cholesterol leading in consequence to pore formation & cell permeabilization, alterations in the negatively charged carbohydrate portions on the cell surface.
- Stimulate apoptotic process in tumor cells, usually through its intrinsic pathway non apoptotic processes were also involved such as cell cycle arrestment, autophagic cell death stimulation, inhibiting of metastasis and cytoskeleton disintegration[16,17]

Saponin performed its antifungal activity by the interaction with sterol membrane of *C. albicans* and disrupting the cell wall 's integrity caused the cell death, similar with the mechanism action of nystatin

Triterpene glicoside content has also been known to have immunomodulatory property on macrophage for the response to infection[13,15] , thus it can be other mode of mechanism of antifungal respond. Further in vivo study need to be conducted to explore this he potential use of the extract as antifungal agent

CONCLUSION

Stichopus hermanii extract had the antifungal effect against *Candida albicans*

Acknowledgement :
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THE ANTIFUNGAL EFFECT OF *Stichopus hermanii* EXTRACT TO *Candida albicans* IN VITRO

Kristanti Parisihni[1,2], Syamsulina Revianti[1,2], Delianis Pringgenies[3]

[1] Hang Tuah University, Surabaya, Indonesia
[2] PhD Student of Airlangga University, Surabaya, Indonesia
[3] Diponegoro University, Semarang, Indonesia

BACKGROUND: Sea cucumbers have long been used for food and folk medicine in the communities of Asia. Regarding to the bioactive compound, some species of sea cucumber have been known to have the biomedical properties as antifungal agent. Oral candidiasis is the most common fungal infection in oral cavity caused by *Candida albicans.* An antifungal agent of natural resource will add the great value on the therapy of oral disease. In this preliminary study, golden sea cucumber (*Sticophus hermanii*) was examined its possible antifungal activity towards *Candida albicans* in vitro.

OBJECTIVE: The aim of this study was to examine the antifungal effect of *Stichopus hermanii* extract to the growth of *Candida albicans*

MATERIAL AND METHOD: The study was an experimental laboratories research with post test only control group design. Three concentration of *Stichopus hermanii* methanolic extract : 20 mg/mL, 40 mg/mL, 80 mg/mL, were tested its antifungal effect against *Candida albicans* by disk diffusion method. The treatment groups were compared to Nystatin oral solution 100.000 IU/ml as positive control and DMSO 1% as negative control. The antifungal effect was examined by measure the diameter of the clear zone around the disk. Data was analyzed by Anova, followed by LSD test.

RESULT: The result of this study showed the clear zone around the disc of *Stichopus hermanii* extract in all concentrations. It had been proved that antibacterial action of extract *Sthichopus herrmanii* could inhibit the growth *Candida albicans* ($p < 0.05$). The largest diameter of the clear zone around the disc was in the concentration of 80 mg/ mL.

CONCLUSION: *Stichopus hermanii* extract had the antifungal effect against *Candida albicans*. Further in vivo study need to be conducted to explore the potential use of the extract as antifungal agent.

Keywords : *Stichopus hermanii,* antifungal, *Candida albicans*

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INTRODUCTION

Indonesian sea possess many species of sea cucumber, *Sticophus hermanii* is the one of the popular ones[1]. Instead of its economical used as meal consume, it was reported in some research that *Sticophus hermanii* as some other species of sea cucumber proved to have some medical properties[2,3]

Therapeutic properties and medicinal benefits of sea cucumbers can be linked to the presence of a wide array of bioactives especially triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan (GAGs), sulfated polysaccharides, sterols (glycosides and sulfates), phenolics, cerberosides, lectins, peptides, glycoprotein, glycosphingolipids and essential fatty acids. Generally, most species of sea cucumber share the same bioactive compound mentioned above but in different level contain[4,5,6].

Candidiasis is the most common fungal infection in oral cavity, its prevalence raised specially along with the raise prevalence of HIV-AIDS[7,8]. A natural source of antifungal agent could become the novel alternative solution in therapy of oral candidiasis .

Extract of sea cucumber has been known to have antibacterial and antifungal properties. *Sticophus hermanii* extract has been proved to have antibacterial effect against Gram positive and negative bacteria : *Escherchia coli, Pseudomonas* sp., *V.vionivica* , *Staphylococcus aureus, Streptococcus mutans*[6,9,10,11]*Holothuria atra* , *Holothuria scabra* and some holothurians species have antifungal action to *Candida albicans* , *Aspergillus sp*[11,12,13]. This study was aimed to examine the antifungal effect of *Stichopus hermanii* extract to the growth of *Candida albicans*

Kingdom	: *Invertebrata*
Phylum	: *Echinodermata*
Class	: *Holothuroidea*
Ordo	: *Aspidochirotda*
Family	:*Sticophoidae*
Genus	: *Sticophus*
Species	: *Sticophus hermanii*

MATERIAL AND METHODS

This study is an experimental laboratory research with post test only control group design. Methanolic extract of *Sticopus hermani* was be tested its antifungal activity to *Candida albicans* by disc diffusion method.

Extract preparation

Adult sea cucumber weight 100-250 gr were collected from Karimunjawa coastal, washed by running water to clean the dirt, immersed in fresh water for one night to remove the salt and adherent parasite. Sea cucumber then splitted, the inner abdomen were removed then cleaned and washed, so only the flesh of the body proceed to next process.

Thirty five (35) sea cucumber sample were cut into small pieces of 3-10 cm, the wet weight then measured then dried up in solar dryer for 3-4 days to reduce the water content. The dried sea cucumber then cut into smaller pieces of 1 cm, mashed by blender the the weight were measured and ready for the maceration process.Two hundred and fifty (250) gram mashed dry sea cucumber sample immersed until soaked in 500 mL methanol solvent for 24 hour at room temperature, then filtered with filter paper to separate filtrate and residue.

Residue then reimmersed in 500 mL methanol solvent for 24 hour, again filtered with filter paper to separate filtrate and residue, resulted in maceration filtrate with the ratio of 250 gram sample / 1000 mL solvent (1:4 w/v).

Methanol (polar) filtrate got homogenized with 1000 mL hexane solvent (non polar) then performed partition with separatory funnel the each of the filtrate layer of methanol and hexane solvent were separated.

Methanol (polar) filtrate then got re-homogenized with 1000 mL chloroform solvent (semi polar), performed partition with separatory funnel the each of the filtrate layer of methanol and chloroform solvent were separated.Each filtrate were separated by its solvent with rotary evaporator until extract produced. The evaporated extract then placed in the vial and stored in -70^{0}C.

Fungal suspension preparation

*Candida albicans*were cultured in Sabouraud dextrose agar, suspensión were prepared by inoculating one single loop of fungal colony to Sabouraud broth médium, incubated in 37^{0}C for 24 hour. Candida suspensión was adjusted its turbidity to stándard McFarland 0,5 by nephelometer Phoenix.

Antifungal activity testing by disk diffusion method. The samples were divided into 5 groups each consisted of 6 samples i.e : positive control was given nystatin oral solution 100.000 IU, negative control was given DMSO 1%, treatment group were given *Sticophus hermanii* extract with concentration of 20%, 40% and 80%. Antifungal activity test was performed by disk diffusion method on Mueller Hinton agar.

Fungal supension of *Candida albicans*equal to 0,5 McFarland was swabbed inoculated onto Muller Hinton agar plate. Sterile paper disks were immersed for 15 second into each contrentation of extracts for treatment groups, for control negative groups in DMSO 1%, each, and for the positive control group in nystatin oral solution, then put on

to Muller Hinton agar, gently pressed for a while and leave, incubated in 37^0C for 2x24 hour.

The clear zone around the disk showed inhibition effect to the growth of *Candida albicans.* Diameter of inhibition zone was measured with digital caliper.

RESULT

All the treatment groups and positive control showed the inhibition zones around the disk , but not the negative control as shown in fig 1.

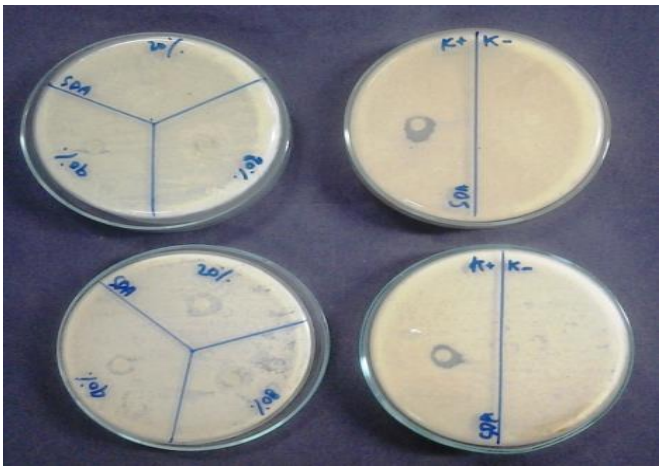


Fig 1. The inhibition zone on disk diffusion method of antifungal activity of *Sticopus hermanii* extract to *Candida albicans*

Table 1 The averagediameter of inhibition zone of *Sticophus hermanii* extracts on serial concentration to *Candida albicans* compared to control groups (stated in mm).

Group	Mean	Std. Deviation
Negative Control	11,4833	1,10306
Positive Control	5,5740	,01242
Stichopus Hermanii 20mg/ml	9,8440	2,74243
Stichopus Hermanii 40mg/ml	9,9027	,81027
Stichopus Hermanii 80mg/ml	10,1360	,76288

Fig 2 The graphic of inhibition zone of *Sticophus hermanii* extracts on serial concentration to *Candida albicans*compared to control groups

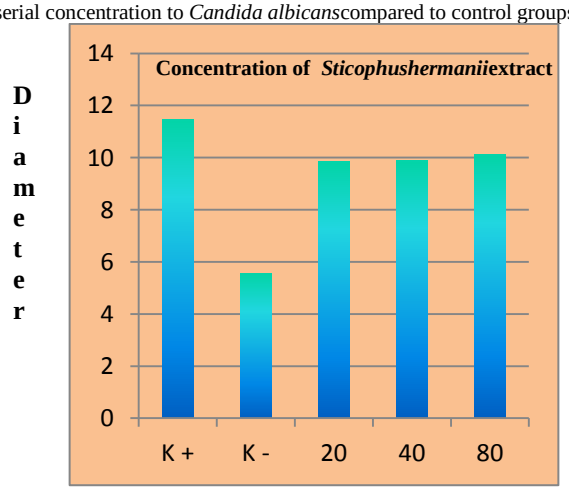


Table 2. ANOVA and LSD test summary of inhibition zone of *Sticophus hermanii* and *Holothuria atra* extracts on serial concentration extracts to *Porphyromonas gingivalis* compared to control groups

(I) Group	(J) Group	Sig.
Negative Control	Positive Control	,000*
	Stichopus Hermanii 20mg/ml	,002*
	Stichopus Hermanii 40mg/ml	,003*
	Stichopus Hermanii 80mg/ml	,011*
Positive Control	Stichopus Hermanii 20mg/ml	,000*
	Stichopus Hermanii 40mg/ml	,000*
	Stichopus Hermanii 80mg/ml	,000*
Stichopus Hermanii 20mg/ml	Stichopus Hermanii 40mg/ml	,910
	Stichopus Hermanii 80mg/ml	,573
Stichopus Hermanii 40mg/ml	Stichopus Hermanii 80mg/ml	,652

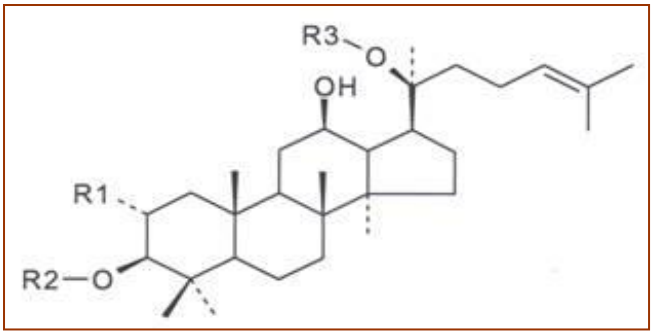
All treatment groups in all concentrations showed inhibition zones but less than nystatin as positive control. Further statistical analysis by two-way ANOVA test and LSD multiple comparison test at 5% significance level showed the significant difference on all concentration of *Sticophus hermanii* extract compared to negative and positive control group ($p<0,05$).

DISCUSSION

Result of antifungal sensitivity test showed inhibition zone in all treatment group and in control positive group, means that extract of *Sticophus hermanii* has antifungal effect to *C. albicans in vitro* The largest diameter of inhibition zone of treatment group was in te concentration of 80 mg/mL but still less than in the control group of *Nystatin (p< 0,05)*

Nystatin is a polyene antifungal drug to which many molds and yeasts are sensitive, including Candida spp, used as the positive control for it's the common topical antifungal agent therapy on oral candidiasis. Nystatin exerts its antifungal activity by binding to ergosterol found in fungal cell membranes. Binding to ergosterol causes the formation of pores in the membrane. Potassium and other cellular constituents leak from the pores causing cell death[7,8].

Sea cucumber extract have been known to have the antifungal property, assumed to be related to its content of alcaloid, saponin and triterpen glycoside[3,,12,13,14]



Saponin were identified in the content of sea cucumber extract[2,14]. It is secondary metabolites of glycosidic nature widely distributed in higher plants and marine invertebrates resulted as the defend mechanism also has the biological properties i.e. the ability to lyse erythrocytes or to foam. It form complexes with cell membrane cholesterol leading in consequence to pore formation & cell permeabilization, alterations in the negatively charged carbohydrate portions on the cell surface. It also could stimulate apoptotic process in tumor cells, usually through its intrinsic pathway, non apoptotic processes were also involved such as cell cycle arrestment, autophagic cell death stimulation, inhibiting of metastasis and cytoskeleton disintegration. [16,17]

Saponin performed its antifungal activity by the interaction with sterol membrane of *C. albicans* and disrupting the cell wall 's integrity caused the cell death, similar with the mechanism action of nystatin

Sticophus hermanii have been extracted by methanolic extract[14]. The antibacterial compound of sea cucumber assumed to be polar for it is dissolved in methanol solvent and have been proven to have the antibacterial, antifungal and cytotoxic agent on some studies[2,5,9,14] In this study, *Sticophus hermanii* extract was examined as the whole extract, probably the optimum content of have not been explored and performed its optimal antibacterial activity. After the methanolic extraction process, the extract was diluted in concentrations with the solvent of DMSO 1% to prevent cytotoxicity. By this dilution, probably the saponin optimum content of have not been explored and performed its optimal antifungal activity. In previous research of other sea cucumber species, it was stated that *Holothuria scabra* extract 7 mg/ml in methanol solvent had proved its antifungal activity against. *Candida albicans.*

Triterpene glicoside content has also been known to have immunomodulatory property on macrophage for the response to infection[13,15] , thus it can be other mode of mechanism of antifungal respond but cannot be examined in in vitro study. Further in vivo approach need to be conducted to explore this he potential use of the extract as antifungal agent.

CONCLUSION

Stichopus hermanii extract had the antifungal effect against *Candida albicans*. Further in vivo study need to be conducted to explore the potential use of the extract as antifungal agent.

Acknowledgement :

This research was supported by a grant from Fundamental Research Program, funded by Ministry of Education and Culture Indonesia.

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