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Antibacterial Activity Extract Hoya Carnosa Leaves, Chloramphenicol 1% and Ciprofloxacin against *Staphylococcus aureus* and *Pseudomonas aeruginosa* that caused benign type Chronic Suppurative Otitis Media (Disc Diffusion Method)

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ABSTRACT

_____The objective of the study was to screening and compare anti bacterial activity of *Hoya carnosa* leaves extract, Chloramphenicol 1% and Ciprofloxacin against bacteria *Staphylococcus* and *Pseudomonas aeruginosa* that cause benign type Chronic Suppurative Otitis Media (CSOM). *Hoya carnosa* leaves extract, Chloramphenicol 1 % and Ciprofloxacin were subjected to screening and compare their antibacterial activity against two strains of bacteria species, *Staphylococcus aureus, Pseudomonas aeruginosa* that taken from culture ears secretions of patients active benign type of CSOM. The study using standard protocol Disc Diffusion Method (DDM). Antibacterial activity were assessed by the presence or absence of inhibition zones and minimal inhibitor capacity (MIC) values. Ciprofloxacin had the most powerful bacterial activity in Disc Diffusion Methods (DDM) with mean rank 13, then Chloramphenicol with mean rank 8 and the weakest is the *Hoya carnosa* leaves extract with mean rank 3. There significant differences among antibacterial activity of *Hoya carnosa* leaves extract, Chloramphenicol 1% and topical Ciprofloxacin against two strains of bacteria species, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Keywords: *Hoya carnosa*, chronic suppurative otitis media.

INTRODUCTION

Chronic suppurative otitis media (CSOM) is a chronic infection of middle ear. *Pseudomonas aeruginosa* is the major bacteria found in secret of CSOM followed by *Staphylococcus aureus.* [1,2,3,4,5] The major complication of CSOM are growth of cholesteatoma and progression of disease to intracranial that developed to death. [4]

Study of epidemiology found that the highest prevalence of CSOM at Alaska, Canada, Greenland, American Indians and Aborigin Australia at 7-46 %.[6] The prevalence of CSOM in South Asia is 1,4-7,8 % .[7] In Indonesia, the prevalence of CSOM at 2004 is 3,1 %.[8]

Many effort was doing to combat CSOM. The problem still exist are few choice of topical anti microba, ototoxicity, biofilm growing up, and resistance of microba with some antibiotic. Study by Lee found that there are tendency of bacterial *Pseudomonas aeruginosa* resistance and decrease of sensitivity with some antibiotic like aminoglycoside, cephalosporin, penicillin, imipenem and quinolone.[9] Ototoxicity also still a major problem when selecting topical antibiotic that suitable for CSOM.

Route of choice for antibiotic in treating CSOM is topical application. Poor vascular condition of middle ear mucosa make sistemic antibiotic cannot achieve MIC after application.[4] Couzos et al proof that topical ear drop most effective in treat CSOM.[10] Quinolon still the drug of choice for CSOM. Unfortunately quinolon stil expensive. Vlastarakos et all found there are resistancy of *Pseudomonas aeruginosa* about 18 %.[11,12] Limitation of antibiotic ear drop that safe for middle ear and high cost for selective antibiotic especially in developing country like Indonesia make scientist thinking other option that still safe and suitable for treating CSOM such as traditional medicine.

The rise of traditional medicine industry in Indonesia and the demand for quality products by the public, cooperation with academic institutions to prove the efficacy and product standardization needs to be done through research. More than 90% of these products are still based on empirical benefits without preclinical proof.

Extract of *Hoya carnosa* is an extract from *Hoya carnosa's* leaves, where in Bali, they has been known with local name " don tebel-tebel", which means the leaves that are thick. *Hoya carnosa* leaves also often called "daun curek" because empirically believed could be used to treat the middle ear diseases as chronic suppurative otitis media (CSOM). Knowledge about the benefits of *Hoya carnosa* as a plant producing ingredients required to be disclosed and disseminated so that knowledge can be utilized for the benefit of mankind or other living creatures.

Table 1: Average Measurement of Inhibitant Zone Flows of Hoya carnosa leaves extract, Chloramphenicol 1% and topical Ciprofloxacin, on the growth of Staphylococcus aureus and Pseudomonas aeruginosa bacteria

Treatment	Σ Diameter of inhibition zone(mm)	
	Staphylococcus aureus	***Pseudomonas aeruginosa***
Hoya carnosa	15,10	11.95
Chloramphenicol	33,40	33,40
Ciprofloksasin	35,70	36,80
Control (-)	0	0

Utilization of *Hoya carnosa* as a drug varies from its use as a medicine for lacerations and burns, swelling, ulcers, bruises, some types of skin diseases caused by microorganisms such as scabies, insect bites and abdominal pain.[13,14] For CSOM disease, has never been done, thus this study aimed to screening and compare the anti bacterial activity of Hoya carnosa leaves extract, Chloramphenicol 1% and Ciprofloxacin against *Staphyloccocus aureus* and *Pseudomonas aeruginosa* in vitro.

MATERIALS AND METHODS

Methods

This study was using laboratory experimental research design in-vitro namely completely randomized design (CRD). This study compares the anti bacterial activity of *Hoya carnosa* leaves extract, chloramphenicol 1% and topical ciprofloxacin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and repeated five times.

Bacteria and reagents

Pseudomonas aeruginosa and *Staphylococcus aureus* were obtained from isolate clinic patient with benign type of CSOM from the population in the village of Abang, Abang sub-District, Karangasem District, Bali Province which then created the culture and identification of bacteria at the microbiology Udayana University-Sanglah Hospital. Chloramphenicol used were chloramphenicol ear drop 1 %, ciprofloxacin used

Table 2: Average Measurement of Inhibitant Zone Zone of Hoya carnosa leaves extract, Chloramphenicol 1% and topical Ciprofloxacin, on the growth of Staphylococcus aureus and Pseudomonas aeruginosa bacteria, based on "Davis and Stout" criteria

Treatment	Σ Diameter of inhibition zone(mm)	
	Staphylococcus aureus	***Pseudomonas aeruginosa***
Hoya carnosa	Strong	Strong
Chloramphenicol	Very strong	Very strong
Ciprofloksasin	Very strong	Very strong
Control (-)	Weak	Weak

were ciprofloxacin ear drop. Mueller Hinton agar used for disc diffusion method.

Selection of plant material

Hoya carnosa leaves with local name "Daun Tebel-tebel or Daun Curek", traditional claims act as anti bacterial for cure CSOM.

Preparation of plant extract

The extracting process consists of extracting and evaporating processes. In the extraction process, *Hoya carnosa* leaves is already in the form of powder wrapped with filter paper and then inserted in a glass of extraction. The extraction was carried out using 96% ethanol (technical ethanol) solvent and was left submerged for +3 days. While the evaporation process using a rotary evaporator. Evaporation process is done until the volume of extraction is reduced and become thick. The evaporation result is stored in the vapor plate then oven for +2 hours at 80 Celcius degree to evaporate the remaining solvent, so we get 100% *Hoya carnosa* leaves extract. The extract is then weighed with an analytical balance.

Preparation of Bacterial Suspension Test

Prior to use, the test bacteria were first confirmed by Gram staining, catalase test and coagulase test. The test bacterial suspension concentration in this study was 106 CFU / ml. *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria were inoculated on Nutrient Broth then incubated at 37-37.5 ° C for 18-24 hours. Furthermore, the seedlings are standardized by using the *Mc Farland* method by equating the turbidity with a standard solution of 0.5 *Mc Farland* with dilution using medium nutrient broth so that the obtained bacterial concentration of 106 CFU / ml.

Table 3: Result of data analysis in normality test

Tests of Normality							
Drug		Kolmogorov-Smirnova			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Staph	Chloramphenicol	0.367	5	0.026	0.684	5	0.006
	Hoya carnosa	0.473	5	0.001	0.552	5	0
	Ciprofloxacin	0.349	5	0.046	0.771	5	0.046
Pseudo	Chloramphenicol	0.367	5	0.026	0.684	5	0.006
	Hoya carnosa	0.473	5	0.001	0.552	5	0
	Ciprofloxacin	0.367	5	0.026	0.684	5	0.006

Table 4 : Results of comparative analysis of anti bacterial activity from *Hoya carnosa* leaves extracts, chloramphenicol 1% and topical ciprofloxacin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Drug		N	Mean Rank
Staph	Chloramphenicol	5	8
	Hoya carnosa	5	3
	Ciprofloxacin	5	13
	Total	15	
Pseudo	Chloramphenicol	5	8
	Hoya carnosa	5	3
	Ciprofloxacin	5	13
	Total	15	

Diffusion Test

The sterile lidi cotton was inserted into a tube containing the bacterial suspension, then the sterile lid cotton was etched on the Mueller Hinton

Tabel 5: Result of "Mann & Withney" test of anti bacterial activity of *Hoya carnosa* leaves extract, Chloramphenicol 1% and topical Ciprofloxacin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

	Staph	Pseudo
Chi-Square	12.939	12.963
Df	2	2
Asymp. Sig.	0.002	0.002

agar (MHA)medium and incubated at 37 ° C for 24 hours. The disc paper is placed on an MHA medium containing test bacteria, then drips 5 ¼l of leaves-thick leaves extract (Hoya carnosa) with concentration of 80%, and 100% w / v, incubated at 37 ° C for 24 hours.[15] The incubation results will show the presence of test bacterial colonies and clear zones around the well, indicating the inhibitory effect of the test solution on the test bacteria. The existing transparent zone is a drag zone measured using a sliding range. Observation Parameter the inhibitory zone formed at 24 hours after the test, was measured and compared with a positive control of Chloramphenicol 1% drops of the ear. Clear areas are an indication of bacterial susceptibility to antibiotics or other antibacterial agents used as test materials expressed to the width of the inhibitory zone diameter.[16] The diameter of the inhibitory area is measured in millimeters (mm) using a scaled threshold by means of the overall diameter minus 7 mm diameter well.

Statistical Methods

Data is presented descriptively in tables containing the type of bacteria in various concentrations. The antibacterial activity of *Hoya carnosa* leaves extract, Chloramphenicol 1% and ciprofloxacin, each of which was incubated with a solution of bacteria in various concentrations.

Data from the testing activity of *Hoya carnosa* extracts against the diameter of inhibition zone growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were analyzed statistically using Shapiro Wilk test to determine the normality and homogenity of data. The results obtained are the data that not normally distributed, so the analysis we will used is non-parametric statistical, Kruskal Wallis test. To determine the differences in leaves extracts for antibacterial activity of *Hoya carnosa* leaves extract on the growth of bacteria carried by Mann and Whitney test.

RESULT AND DISCUSSION

Data results from comparisons test of anti bacterial activity of *Hoya carnosa* leaves extracts, chloramphenicol 1% and topical ciprofloxacin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, normality test after the treatment was tested by using the Shapiro-Wilk test. The results showed that the data were not normally distributed because the *Staphylococcus* and *Pseudomonas* almost all have a significance value of 0.001 (<0.05) as presented in Table 3, so the statistical analysis performed is by using the non parametric test, Kruskal Wallis test.

Comparative analysis of anti bacterial activity test data of *Hoya carnosa* leaves extract, Chloramphenicol 1% and Topical Ciprofloxacin to *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria was performed using "Mann & Withney" test with the result obtained; There was a difference where in both bacteria it was found that Ciprofloxacin had stronger anti bacterial activity compared with Chloramphenicol and *Hoya carnosa* leaves extract where the mean rank of Ciprofloxacin was 13, the second was Chloramphenicol which was 8 and the weakest of the *Hoya carnosa* leaves extract 3, Presented in table 4 and the difference is significant because the significant value of these four bacteria P.value 0.02 (<0.05), as presented in table 5.

Pseudomonas aeruginosa is a gram negative bacteria and aerob obligat. This bacteria found commonly in the environment. *Pseudomonas aeruginosa* also resistance with some antibiotic. Study of the *Hoya carnosa* extract against *Pseudomonas aeruginosa* found that the formation of small diameter inhibition zone thought to be caused by the compound on non polar extracts that are difficult to diffuse in the agar medium used. Among these chemical compounds, there are flavonoids, saponins, tannins and alkaloids. The level or the extent of the extracts activity is on a paper disc depends on the diffusion rate of the extract on the media and the potential of the extract. The extract has a high bioactivity potential, it may has a physical properties which is difficult to diffuse in the media so that the diameter of the bacteria inhibition is in small size. While ciprofloxacin and Chloramphenicol has a strong anti bacterial activity because it has undergone a process of making antibiotic drug with a binding agent added so that the antibacterial effect will increasingly strong and comprehensive.

In this study, the solvents used is ethanol extract. It is a universal solvent, so that the polar

compounds are also interested in the extract. This led to the expected activity of the anti bacterial compounds is not optimal, because it works synergistically with the activity of the polar compound contained in extracts of *Hoya carnosa*. Qualitatively, inhibitory power of *Hoya carnosa* extract is around 10 mm - 20 mm, the data is ordinal (ranking), which has a long range. To obtain the optimal inhibition of anti bacterial, the compounds must be done an identification for flavonoid which is the main antibacterial substance on the *Hoya carnosa* leaves.

In order for this study can be utilized by the community, we need to do some in vivo animal experimental trial, toxicity and side effects which then it can be continued with clinical trial. If the Hoya carnosa leaves extract has been through the stages of clinical trials, *Hoya carnosa* leaves extract will be able used by the public as an alternative eardrops from herbal on active benign type of CSOM.

CONCLUSION

From the sensitivity test, we found that Ciprofloxacin had the most powerful anti bacterial activity, then Chloramphenicol and the weakest is the extract of *Hoya carnosa* leaves extract.

REFERENCES

1. Prakash, R., Juyal, D., Negi, V., Pal, S., Adekhandi, S., Sharma, M., & Sharma, N. Microbiology of Chronic Suppurative Otitis Media in a Tertiary Care Setup of Uttarakhand State, India. *North American Journal of Medical Sciences,* **5**(4), 282–287 (2013). http://doi.org/10.4103/1947-2714.110436
2. Maji, P., Chatterjee, T., Chatterjee, S., Chakrabarty, J. & Mukhopadhyay, B. 2007. The investigation of bacteriology of chronic suppurative otitis media in patients attending a tertiary care hospital with special emphasis on seasonal variation. *Indian journal of otolaryngology and head & neck surgery;* **59**: 128-131 (2007).
3. Renukananda, G.S., Santosh, U.P., Nitha, M.G. Topical VS combination ciprofloxacin in the management of discharging chronic suppurative otitis media. 2014.
4. Morris, P. Chronic suppurative otitis media. Bmj clin evid, 2001, (2012)
5. Sharma, K., Aggarwal, A. & Khurana, P. M. Comparison of bacteriology in bilaterally discharging ears in chronic suppurative otitis media. *Indian j otolaryngol head neck surg,* **62**: 153-7 (2010).
6. Verhoeff, M., Van der veen, E. L., Rovers, M. M., Sanders, E. A. & Schilder, A. G. Chronic suppurative otitis media: a review. International *journal of pediatric otorhinolaryngology,* **70**: 1-12 (2006).
7. Helmi. 2005. Chronic suppurative otitis media. In Chronic Suppurative Otitis Media: basic knowledge, medical therapy, mastoidectomy, tympanoplasty. Jakarta: Faculty of Medicine, University of Indonesia; 76-92
8. Anggraeni, R., Hartanto, W.W., Djelantik, B., Ghanie, A., Utama, D.S., Setiawan, E.P., Lukman, E., Hardingsih, C., Asmuni, S., Budiarti, R., Rahardjo, S.P., Djamin, R., Mulyani, T., Mutyara, K., Carosone-Link, P., Kartasasmita, C.B., Simoea, E.A. Otitis Media ini Indonesian Urban and Rural School Children. *Pediatr Infect Dis J.* **33**(10):1010-15 (2014). http://doi.org/10.1097/INF.0000000000000366
9. Lee, S. K., Park, D. C., Kim, M. G., Boo, S. H., Choi, Y. J., Byun, J. Y., Park, M. S. & Yeo, S. G. Rate of isolation and trends of antimicrobial resistance of multidrug resistant pseudomonas aeruginosa from otorrhea in chronic suppurative otitis media. *Clin exp otorhinolaryngol,* **5**: 17-22 (2012).
10. Couzos, S., Lea, T., Mueller, R., Murray, R. & Culbong, M. Effectiveness of ototopical antibiotics for chronic suppurative otitis media in aboriginal children: a community-based, multicentre, double-blind randomised controlled trial. *Med j aust,* **179 :**185-90 (2003).
11. Vlastarakos, V. V., Nikolopoulos, T. P., Maragoudakis, P., Tzagaroulakis, A. & Ferekidis, E. Biofilms in ear, nose, and throat infections: how important are they? *The*

laryngoscope, **117**: 668-73 [9] (2007).

12. Bluestone, C. D. Efficacy of ofloxacin and other ototopical preparations for chronic suppurative otitis media in children. Pediatr infect dis j, 20: 111-15; discussion 120-22 (2001).
13. Rahayu S.2001. The genetic diversity of Hoya (Asclepiadaceae) from Sumatra (Thesis). Bogor: Post-graduate program. Bogor Agricultural Institute Burton, C.M.2007.Hoyas in medicine. *The Hoyan* **18**(3):15-17
14. Janak Kishore. Isolation, identification & characterization of proteus penneri-a missed rare pathogen. Departement of microbiology, Sanjay Gandhi Post- Graduate Institute of Medical Sciences. *Lucknow Indian J Med Res* **135**: 341-5 (2012).
15. Vandepitte, Basic laboratory prosedure for clinical bacteriology. Second Edition. EGC. Jakarta (2005).