

Ethanol Extraction of *Hoya Carnosa* Leaves Improved Stroma of Middle Ear Epithelium Infected by *Pseudomonas Aeruginosa*

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Hoya carnosa leaves or tebal-tebel leaves are known by the Balinese people in the countryside to treat middle ear infections in the medical language, otitis media or "curek" in the Balinese language. The study is true experimental randomized posttest-only control group design to evaluate the impact of ethanol extract sample as antibacterial. As antibacterial, the extract has an impact on the thickness of stroma middle ear epithelium with otitis media due to *Pseudomonas Aeruginosa*. The result showed average mean thickness stroma in the treatment group lower than controls.

Keywords: Ethanol extract, *Hoya carnosa*, stroma, middle ear, *Pseudomonas aeruginosa*.

Otitis media is a disease that is still often found in society especially in developing countries like Indonesia. Otitis media that occurs chronically is mainly caused by *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is a gram-negative bacterium with the ability to produce biofilms which increases the potential for resistance to some antibiotics. *Pseudomonas aeruginosa* is also a malignant gram-negative bacterium because the enzyme produced is osteolytic which causes deeper damage to the structure of the middle ear. Handling this infection is quite difficult and tends to cost a lot. The main choice of treatment for otitis media due to *Pseudomonas aeruginosa* is topical.

The choice of topical antibiotics is limited and quite expensive. Research conducted by Lee *et al.* (2012) between 2001 and 2008 showed a tendency to increase resistance to *Pseudomonas aeruginosa* bacteria and decrease sensitivity to aminoglycoside antibiotics, cephalosporin, penicillin, imipenem and quinolones¹. Vlastarakos *et al.* (2007) also found resistance of *Pseudomonas aeruginosa* by 18% to the quinolone class². Jang *et al.* (2007) found an association between antibiotic resistance and biofilm formation by *Pseudomonas aeruginosa* in CSOM³.

During otitis media occur transition middle ear epithelium from single layer simple

squamous epithelial cells and a rudimentary stroma to a pseudostratified respiratory epithelium with extensive stroma. Normally middle ear mucosa when otitis media histologically showed significant thickening at day 2 and resolution at day 10.

Indonesia with diverse medicinal plants is very potential to be studied for its effectiveness as an antibacterial in the handling of otitis media due to the *Pseudomonas aeruginosa* bacteria. Balinese people in ancient times empirically used ear drops from the extract of the leaves of *Hoya carnosa*, which is better known as the area “*don tebel-tebel*” which means the leaves are thick or often also called “*don curek*” to treat inflammation of the middle ear in language the area of Bali is called “*curek*”. *H. carnosa* is a species of *Asclepiadaceae*, included in the list of Chinese medicinal plants which contain phenolic components such as phenolic alkaloids, phenolic acids, flavonoids, tannins, phenolic terpenoids, quinone, stilbenes, volatiles and aliphatic components⁴. Most of the contents of the plant are flavonoids⁴. The results of the experimental antibacterial activity of extract showed that with a concentration of 80% and 100% had a strong inhibitory effect on *Staphylococcus aureus*, *Streptococcus sp* and *Pseudomonas aeruginosa*⁵.

It is necessary to do an *in vivo* test to find the ability of *tebel-tebel* leaves as anti-bacterial and anti-inflammatory that show resolution

of middle ear mucosal stroma after infections by *Pseudomonas aeruginosa*.

MATERIAL AND METHODS

This study is true experimental randomized posttest-only control group design to evaluate the impact of ethanol extract sample as antibacterial by improving stroma of middle ear epithelium. This study already has approval from Ethic Committee Udayana University. The sample used in this study were 36 male BalbC mice, having a body weight of 25-30 grams and aged 3-3.5 months, which were maintained during study with standard feed and randomized into treatment and control group. The preparation material for induction of otitis media is *Pseudomonas Aeruginosa* ATCC 9027 from Microbiology Laboratory Udayana University.

Research procedure

The action is carried out aseptically. Mice are anesthetized with ketamine 100 mg/kg BB and xylazine 0.1 mg/kg intramuscularly. The next step mice are placed in a prone position. The tympanic membrane was identified under the help of SHIN-NIPPON OP-2 otology microscope. The intact tympanic membrane and pearl white images without the shadow of an effusion in the middle ear indicate mice is a suitable candidate as a research sample. Next, 0.1 ml of *Pseudomonas aeruginosa*

Table 1. Stroma Thickness

	Group	N	Mean ±Std deviation	t	p
Stroma Thickness	Treatment	18	33.62 ± 14.33	5.83	0.001
	Control	18	67.21 ± 19.83		

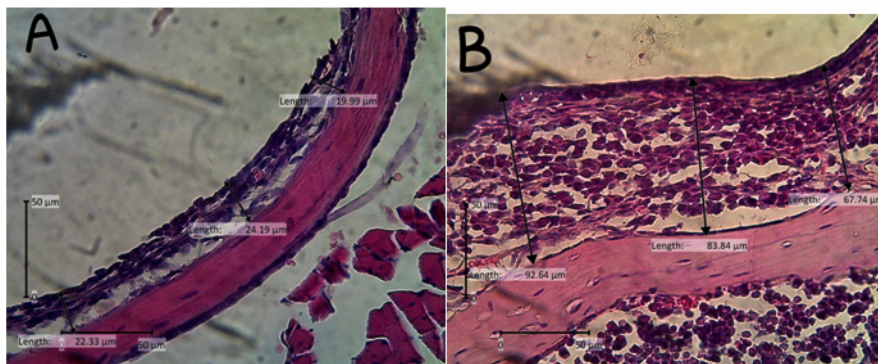


Fig. 1. Treatment group (A) showed thinner stroma and less infiltration of cell immun than control group (B)

solution (CFU) was applied to the right and left tympanic bulla trans tympanic. Mice middle ear was evaluated with otology microscope 48 hours after bacterial inoculation to see the presence of otitis media. The presence of changes in opacity and color tympanic membrane and the image of the effusion in the middle ear indicate the presence of otitis media. Furthermore, the extraction of 0.1 ml *tebel-tebel* leaves was dropped in the right and left tympanic bullae of the treatment group via trans tympanic route. 24 hours after giving the extract, mice euthanized by decapitation technique and the tympanic bullae tissue is taken. Tympanic bullae tissue then processed for Hematoxylin and Eosin staining.

RESULTS AND DISCUSSION

Test of normality data using Shapiro–Wilk. Levene’s test show homogen of data. Independent t- test to check equality of means showed significant differences stromal thickness between treatment and control group.

Histopathological stromal epithelial from treatment group thinner than control. This probably caused by antibacterial activity of the extract on middle ear epithelium that already infected by *Pseudomonas Aeruginosa*. The component of flavonoids, tannin, phenolic and alkaloid work together as antibacterial at multiple site of bacteria and infected tissue make improvement of middle ear stroma.

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REFERENCES

1. Lee, SK, Park, DC, Kim, MG, Boo, SH, Choi, YJ, Byun, JY, Park, MS & Yeo, SG. Rate of isolation and trends of antimicrobial resistance of multidrug resistant *Pseudomonas aeruginosa* from otitis media in chronic suppurative otitis media. *Clin Exp Otorhinolaryngol*, **5**: 17-22 (2012).
2. 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 (2007)
3. Jang, C.-H., Cho, Y.-B. & Choi, C.-H. Structural features of tympanostomy biofilm formation tube in ciprofloxacin-resistant *Pseudomonas aeruginosa*. *International journal of pediatric otitis rhinology*, **71**: 591-595 (2007).
4. Huang, W. & Zhang, H. S. 2008. Traditional Chinese medicinal plants and their endophytic fungi: isolation, identification, and bioassay. [Hkthesesonline \(hkuto\)](http://hkthesesonline.hkuto)
5. Yully, P. 2016. In vitro test of the antibacterial effectiveness of *tebel-tebel* leaf extract (*Hoya carnosa*) on gram-positive and gram-negative bacteria causing benign type active CSOM. Udayana University.