Herbal Extract as An Antibacterial Against Gram Positive Bacteria Causing Dermatitis Complex

I Nyoman Suartha[1*], I GustiKetut Suarjana[2], Luh Made Sudimartini[3], I Made Merdana[4], I Made Dira Swantara[5]

[1]Veterinary Internal Diseases Laboratory;
[2]Veterinary Microbiology Laboratory; [3,4]Veterinary Pharmacology Laboratory
Faculty of Veterinary Medicine Jl PB Sudirman Denpasar
[5]Department of Chemistry, Faculty of Mathematics & Science
Udayana University,Jl PB Sudirman Denpasar 80232
*corresponding author: nyoman_suartha@unud.ac.id

Abstract. Study in order to evaluate the antibacterial effect of herbal extract from leaves of three different species of plant namely neem(Azadirachtaindica A. Juss), gotu-kola(Centellaasiatica), andsoursop (Annona muricata L) against bacteria causing dermatitis complex in dogs have been undertaken. The antibacterial activities of the herbal extract were evaluated using the agar diffusion test with extract at three different concentrations 5%, 10%, and 25%, respectively.Staphylococcus aureus was isolated from the dermatitis complex cases in dogs. The results showed various antibacterial activities of all the herbal extract at all concentration as indicated by the diameter of inhibition haloes produced around the orifice. The average inhibition haloes for each herbal extract concentration was 2.19± 0.53 mm; 4.32± 0.84 mm; 5.86± 0.79 mm, respectively. It can be concluded that herbal extract from the three plants is a potential antimicrobial against agents causing dermatitis complex.

Keywords: **herbal extract, Gram positive bacteria,dermatitis complex, dogs.**

I. INTRODUCTION

The prevalence of skin diseases in dogs in Bali and Indonesia is relatively high [1][2] and majority is caused by Gram positive bacteria such as Staphylococcus spp. As noted in Yayur Weda Unani medication and Homoeopathic [3][4] the use of herbal for medication is well known. Amongst that the use of herbal for controlling antibiotic resistance has been widely applied. Plants such as neem (Azadirachtaindica A. Juss), gotukola (Centellaasiatica), and soursop (Annona muricata L) grew enormously in Indonesia [5]. Several studies have indicated that these plants as a potential antimicrobial agents [6][7][8][9], antioxidant [10][11], antineoplastic [12]; and immunomodulator [5]. This study aimed to evaluate the antibacterial effect of herbal extract from leaves of neem (Azadirachtaindica A. Juss), pegagangotu_kola Centellaasiatica), and soursop (Annona muricata L) against Gram positive bacteria causing dermatitis complex in dogs.

II. RESEARCH METHOD

Isolation and Identification of Bacteria Causing Dermatitis

Isolation and identification of bacteria was performed following the methods described in Carter (1984) with slightly modification. Clinical specimens were aseptically collected by swab the wound area in the skin of dogs. Subsequently, specimens were cultured onto Blood Agar plate and incubated at 370C overnight. Morphological identifications were performed based on the shape, diameter, edge, color, and hemolytic typeof the colonies shown on the Blood Agar plate surface. Further identification including Gram staining, catalase and oxidase test, and coagulation test.

Antimicrobial Test

Ethanol extract of neem (Azadirachtaindica A. Juss), gotu-kola (Centellaasiatica), and soursop (Annona muricata L) was prepared at concentration of 50 mg/mL, 100 mg/mL, and 250 mg/mL solubilized with Tween 80 at 20 mg/ml, respectively. A negative control solution was used, consisting of sterile distilled water and tween 80 (20 mg/mL). Tests were performed with two replicates. Antibacterial activity of each extract was evaluated adapting the technique of plate cylinder diffusion in agar [13]. Lawn cultures of 107 CFU/mL Staph. Aureusadjusted to 0.5 MacFarland scale were prepared on the surface of agar media. Orifices/wells were formed in the agar media and

each well was inoculated with 50 ul of the herbal extract. After preparation, the plates were incubated at 37°C for 24 h. Antimicrobial activity was represented by inhibition haloes that were measured in millimeters. A positive result is considered if haloes of any size were present around the orifice/well. Tests were performed with two replicates.

III. RESULTS AND ANALYSIS

The Gram positive bacteria, Staph. aureus isolated from dermatitis complex cases in dogs is shown in Fig. 1.

Fig 1. The Morphology of Colonies of Suspected Gram Positive Bacteria Causing Dermatitis Complex in Dogs on Blood Agar Plate.

Table 1 showed the results of identification of the Gram positive bacteria based on colony morphological, Gram staining, catalase and oxidase test.Staphylococcus is one of the Gram positive bacteria which frequently isolated as secondary infection in dermatitis cases in dogs [14].

TABLE 1.
ISOLATION AND IDENTIFICATION OF GRAM POSITIVE BACTERIA CAUSING DERMATITIS IN DOGS.

Isolate Code	Sample distribution	Morphology on MacConkey Agar	Gram staining	Catalase test	Oxidase test	Conclusion following sugar test
A	40% (4/10)	Small, smooth edge, convex, white beta-hemolytic.	+	+	+	*Staphylococcus aureus*

Staphylococcus aureus was found highly sensitive to the ethanol extract of leaves of neem(Azadirachtaindica A. Juss), gotu-kola(Centellaasiatica), and soursop (Annona muricata L). Sensitivity was observed at concentration of 5% and significantly different to the concentration of 25%. The average inhibition haloes for each herbal extract concentration was 2.19 ± 0.53 mm; 4.32 ± 0.84 mm; 5.86 ± 0.79 mm, respectively. Results of the diameter of inhibition of the bacteria in vitro are shown in Table 2.

TABLE 2.
ANTIBACTERIAL EFFECT OF ETHANOL EXTRACT OF LEAVES OF NIMBA (AZADIRACHTAINDICA A. JUSS), PEGAGAN (CENTELLAASIATICA), AND SOURSOP (ANNONA MURICATA L)

No	Type of Herbal Extract	Haloes diameter (mm) at Each Concentration of Extract			
		0%	5 %	10%	25%
1	Neem*(Azadirachtaindica)*	0 ± 0.00[a]	4.18 ± 0.26[b]	4.30 ± 0.85[b]	5.12 ± 0.48[c]
2	Gotu-kola*(Centella asiatica)*	0 ± 0.00[a]	4.20 ± 0.86[b]	4.25 ± 0.95[b]	6.20 ± 0.94[c]
3	Soursop *(Annona muricata)*	0 ± 0.00[a]	4.20 ± 0.48[b]	4.40 ± 0.71[b]	6.25 ± 0.96[c]
	Average	0 ± 0.00	2.19 ± 0.53	4.32 ± 0.84	5.86 ± 0.79

Note: different letters towards the column indicate significantly differences (P<0.05)

The extract of neem, gotu-kola and soursop leaveshas several properties such as flavonoid, alkaloids, saponin, and tannins which has the ability to inhibit bacterial growth and motility and to destroy bacterial cell membrane [9] as well as wound healing [15]. Saponin has the ability as antimicrobial as well as antiseptic [16]. Alkaloids have been identified to play a role in disturbing the components of peptidoglycan resulting in the failure of the bacteria to form the cell wall [17][18][9]. Besides ethanol extract, aqueous extract of soursop seed also has antibacterial activity [6]. Essential oils of neem leaves also has some substance that is larvacidal towards Culicoides [19]. Based on the results of this study it is concluded that at low concentration (5%) ethanol extract of neem, gotukola, soursop leaves will inhibit the growth of Staphylococcus aureus the agent of causing dermatitis complex in dogs.

ACKNOWLEDGMENT

The authors would like to express their appreciation to The Ministry of Research & Technology and Higher Education through Udayana University Institute for Research and Community Service for supporting this project under University Superior Grant contract No: 486.40/UN.14.2/PNL.01.03.00/2016.

REFERENCES

[1] Widyastuti, S.K., Sutaridewi, N.M., Utama, I.H. (2012). Kelainan Kulit Anjing Jalanan pada Beberapa Lokasi di Bali. Bul. Vet. Udayana. 4(2): 81-86.

[2] Tjahajati, I., Widiastuti, T.A., Erarindah, E., Prayitno, A.D., Rifqiyanto, L., Hanafi, I. (2014). Macam pasien dan persentase pasien anjing dan kucing yang terinfeksi endoparasit dan ektoparasit yang ditangani di klinik hewan Jogja tahun 2013-2014. Proseding Kivnas ke-13 PDHI. Palembang 23-26 Nopember 2014.

[3] Kumar, V.S., Navaratnam, V. (2013). Nimba (Azadirachtaindica): Prehistory to Contemporary Medicinal Uses to Human Kind. Asian Pac J Trop Biomed. 3(7): 505-514.

[4] Hossen, M.J., Uddin, M.B., Ahmed, S.U.U., Zhi-Ling Y., and Cho, J.Y. (2016). Traditional Medicine/Plants for the Treatment of Reproductive Disorders in Asia Nations. Pak Vet.J. 36(2): 127-133.

[5] Surya, I.G.M.G., Trapika, C., Mustofa, Sholikhah, E.N. (2012). Effect of Pegagan Leave (Centella Asiatica [L]. Urban) Ethanol Extract on IFN-γ Secretion on the Spleen of Balb/C Mice that Infected with Listeria Monocytogenes. International Conference: Research and Application on Traditional Complementary and Alternative Medicine in Health Care (TCAM) June, 22 nd-23rd 2012 Surakarta Indonesia .

[6] Vieira, G.H.F., Mourão, J.A., Ângelo, A.M., Costa, R.A. & Vieira, R.H.S.F. (2010). Antibacterial Effect (In Vitro) Of Moringa Oleifera And Annona Muricata Against Gram Positive And Gram Negative Bacteria. Rev. Inst. Med. Trop. Sao Paulo 52(3):129-132.

[7] Ayini, U., Harnina, S.B., Dewi, T.C. (2014). Neem Leaf Extract Antibacterial Effect (Azadirachtaindica A. Juss) against Vibrio algynoliticus Bacteria In Vitro. Biosaintifika. 6(1): 67-75.

[8] Margaret, A., Yolanda, H., and Wibisono, L.K. (2013). Antifungal Activity of Neem Leaf.

[9] Deshmukh, P., Sharma, R.K., Sharma, V. and Nayak, A. (2016). In Vitro Study on Antibacterial Activity of Moringa Oleifera Leaves. Indian Vet. J. 93 (01):32-33.

[10] Adewole, S.O., Ojewole, J.A.O. (2009). Protective Effects of Annona muricata Linn. (Annonaceae) Leaf Aqueous Extract on Serum Lipid Profiles and Oxidative Stress in Hepatocytes of Streptozotocin-Treated Diabetic Rats. Afr J Tradit Complement Altern Med. 6(1): 30–41.

[11] Anggia, S.W.J.(2015). The Effect of Ethanol Extract of Soursop Leaves (Annona muricata L.) to Decreased Levels of Malondialdehyde. J Majority. 4(3) : 14-18.

[12] Arifianti, L., Sukardiman, Studiawan, H., Rakhmawati, Megawati, L. (2014). Uji Aktivitas Ekstrak Biji Sirsak (Annona muricata L.) Terhadap Sel Kanker Mamalia Secara In Vitro. Jurnal Farmasidan Ilmu Kefarmasian Indonesia,1(2):63-66.

[13] Pieri, F.A., Silva, V.O., Vargas, F.S., Junior, V.V.F., Moreira, M.A.S. (2014). Antimicrobial Activity of Copaiferalangsdorffii Oil and Evaluation of its Most Bioactive Fraction against Bacteria of Dog's Dental Plaque. Pak Vet J. 34(2): 165-169.

[14] Mueller, R.S. (2007). Dermatology for Small Animal Practitioner. Pub. Teton New Media. Ithaca New York.

[15] Biswas, K., Chattopadhyay, I., Banerjee, R.K., Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (Azadirachtaindica). Current Science, 82 (11): 1336-1345.

[16] Purohit, S.K., Solanki, R., Soni, R., Mathur, V. (2013). Evaluation of wound healing activity of ethanolic extract of azadirachtaindica leaves in male albino rats. Asian J. Pharm. Tech. 3 (2): 73-75.

[17] Akhouri, S., Prasad, A., and Ganguly, S. (2013). Moringa oleifera leaf extract imposes better feed utilization in broiler chicks. J. Biol. Chem. Res. 30 (2) : 447-450.

[18] Akhouri, S., Prasad, A., and Ganguly, S. (2014.) A poultry performance enhancer herb: a review. World J.Pharm. &Pharma. Sci. 3 (7):523-525.

[19] Narladkar, B.W., and Shivpuje, P.R. (2015). Herbal Agents for Biological Control of CulicoidesSpp (Diptera:Ceratopongonidae) Indian Vet. J. 92 (5):20-23.