International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Gonad Extracts of *Diadema setosum* as Potential Antibacterial Agent Derived from Wakatobi District Sea Waters Southeast Sulawesi Province-Indonesia

Wa Ode Salma[a*], Asriati[b], Amiruddin Eso[c], Adius Kusnan[d], Adryan Fristiohady[e], Karwika Dwi Saputri Nurdin[f], Muhammad Rahmat Bastaman[g], Mu'afif Nur Abdilah[h], Nabilah Hanun Mudjahidah[i]

[a]*Department of Nutrition Science, Faculty of Public Health, Halu Oleo University, Kendari- Indonesia*

[b-d]*Department of Medical Science, Faculty of Medicine, Halu Oleo University, Kendari- Indonesia*

[e]*Faculty of Pharmacy, Halu Oleo University, Kendari- Indonesia*

[f-i]*Medical Study Program, Faculty of Medicine, Halu Oleo University, Kendari Indonesia*

[a]*Email: waode.salma@uho.ac.id,* [b]*Email: asriatiyusuf@gmail.com,* [c]*Email: amir_fk_uhalu@yahoo.co.id*

[d]*Email: adius_kusnan@yahoo.com,* [e]*Email: adryan_pharm@yahoo.com*

[f]*Email: karwikadwisaputrinurdin@gmail.com,* [g]*Email: rahmatbastaman69@gmail.com*

[h]*Email: muafifabdillah@gmail.com,* [i]*Email: nabilahhanun98@gmail.com*

Abstract

High incidence rates of typhoid fever and urinary tract infections in developing countries are aggravated by the overuse of antibiotics that lead to higher resistance of strains of bacteria, including *Salmonella typhi* and uropathogenic *E. coli* (UPEC). This dilemma instigates recent studies to find alternative drugs derived from marine resources to reduce the resistance of these harmful bacteria through the utilization of bioactive compounds in the gonad of *Diadema setosum*. For that reason, this study was conducted to explore the antibacterial properties of ethyl acetate extract in the gonad of *Diadema setosum* on *S.typhi* and *E. coli* bacteria. Chemical screening of bioactive compounds in the gonad of *Diadema setosum* used ethyl acetate solvent, whereas the antibacterial sensitivity test was conducted by diluting the gonad extracts in 10% DMSO. Preparation of 10% DMSO was made by mixing 10 ml DMSO with a 90 ml aquadest.

---

* Corresponding author.

Each gonad extract of 1 g, 2 g, 4 g, and 8 g was diluted in 10 ml DMSO with the extract concentrations of 10%, 20%, 40%, and 80%, respectively. Results of the chemical screening of the gonad extracts of *Diadema setosum* showed that the gonad extracts contained flavonoids (orange to red color), steroids (greenish color), and saponin was marked in the form of foam for 15 minutes. Meanwhile, results of the culture test proved that ethyl acetate extract of the gonad of Diadema setosum inhibited the growth of *E. coli* and *S. typhi* at 80% concentration classified into high inhibition response with mean inhibition response was 21 mm for *E. coli* and mean inhibition response was 20 mm for *S. typhi* compared to the concentrations of 40%, 20%, and 10 %. It was concluded that the gonad extracts of *Diadema setosum* could be used as a potential novel drug for reducing the resistance of these bacteria. In addition, the gonad of *Diadema setosum* delivers a potential alternative foodstuff to improve health status and prevents the negative effects of these bacterial strains.

Keywords: E. coli; Diadema setosum; gonad; S.typhi; Wakatobi District Sea Waters.

1. Introduction

In view of the global scope, infections due to gram-negative bacteria such as *E. coli* and *S. typhi* remain a major public health concern particularly in developing countries, including Indonesia. These bacteria are commonly transmitted through contaminated water and food in crowded populations with inadequate sanitation [1]. These bacteria result in negative impacts on health that was significantly reducing the quality of life of men and women [2], particularly impoverished populations and susceptible age groups, including children with high infection risk [3].

High incidence rates of typhoid fever are aggravated by the overuse and misuse of antibiotics that lead to higher resistance of *S.typhi* strains on antibiotics [4]. Meanwhile, urinary tract infections (UTIs) are caused by specialized *Escherichia coli* strains referred to as uropathogenic *E. Coli* (UPEC) that globally also resist antibiotics for the last 15 years [5].

As attested in the study of Lindayanti and his colleagues (2014) at the Adam Malik General Hospital in Medan-Indonesia that the antimicrobial resistance patterns of *E. coli* showed variations on antibiotics that include ampicillin, tetracyclin and trimethoprim/sulfamethoxazole (100%), cefotaxime, ceftriaxone, cefpodoxime and ciprofloxacin (78%), and gentamycin and aztreonam (67%) [6]. In a study that investigated the antibiotic resistance pattern of *Salmonella typhi* in typhoid fever patients within 2011-2015 in Tangerang-Indonesia showed that antimicrobial resistance patterns for ampicillin (5.4%), trimethoprim/sulfamethoxazole (8.6%), ciprofloxacin (1.1%), and levofloxacin (3.2%) respectively [7].

Other studies reported various methods in minimizing the resistance of bacteria by utilizing herbal-based and plant-derived products that proved positive effects in reducing bacterial resistance with low side-effects. Meanwhile, recent investigations have been made to discover a novel drug from the extraction of bioactive compounds derived from marine resources, including the gonad of *Diadema setosum* in reducing the resistance of harmful bacteria. A recent study reported that 96% ethanol extracts of the gonad of *Diadema setosum* had potential antibacterial agents [8] and regulation of the body immune response as proven from the increase of

gene expression of FOXP3 in mice BALB/c infected by *S. typhi* [9]. Concerning these reasons, we intend to widely explore the utilization of gonad of *Diadema setosum* by assessing ethyl acetate extract of the gonad of *Diadema setosum* as an antibacterial bioactive compound on *S.typhi* and *E. coli*.

2. Materials and Method

2.1. Procedure of Extraction and Dilution of the Gonad Extracts of Diadema setosum

Extraction of the gonad of *Diadema setosum* was conducted at the Pharmacy Laboratory, Faculty of Pharmacy of the Halu Oleo University, Kendari-Indonesia. The maceration method was used for the extraction of the gonads using 96% ethyl acetate solvent that referred to the previous method [10]. In brief, air-dried samples of the gonad (50 g) were weighed and were mixed with 96% ethyl acetate solvent with a ratio of 1:3 and were vibrated using the shaker with 180 rpm for 24 hours. The extracts were then filtered using Whatman no. 1 filter paper. The resulted extracts were evaporated using a vacuum rotary evaporator at 37 - 40 ^{o}C. Meanwhile, an antibacterial activity test was conducted at the Microbiology Laboratory of Medicine Faculty of the Halu Oleo University, Kendari-Indonesia. The process of antibacterial activity test was performed using dilution in which the extract concentrations were determined by the preliminary test that referred to the procedure of the previous method [8]. Dilution of the gonad extracts used 10% DMSO. Preparation of 10% DMSO was made by mixing 10 ml DMSO with a 90 ml aqua dest. Extracts of 1 g, 2 g, 4 g and 8 g were diluted in 10% DMSO (10 ml) until achieved concentrations at 10%, 20%, 40% and 80%. After that, the extracts in the vials were wrapped with plastics and were incubated for 24 hours. Chemical screening of bioactive compounds was done according to the procedure of the previous method [11].

2.2. Inoculation and Preparation of Suspensions of S. typhi and E. coli

Preparation of nutrient agar media for bacterial inoculation referred to the procedure as described in the previous methods [12,13]. The prepared nutrient agar media (5 ml) were put into three vials and were sterilized in the autoclave at 121°C for 15 minutes. After sterilization, the vials were placed in an inclined position until the nutrient agar media become solid [14]. The test bacteria were then stricken in zigzag lines on nutrient agar media, and the multiplication of bacterial growth in the nutrient agar media was subsequently observed after incubation for 24 hours at 37.5 ^{o}C with pH 6-8, according to the procedure of the previous method [15]. Thereafter, the inoculated test bacteria were take using sterile micropipettes and were suspended in 2 ml NaCl (0.9 %) until formed turbidity by adjusting the turbidity level of the suspension to a 0.5 McFarland standard. The same procedure was done for other strains of the bacteria according to the procedure of the previous method [12].

2.3. Preparation of the Control Solution

The positive control was made by diluting 1 g ceftriaxone in10 ml aqua dest. Dilution of 1 g ceftriaxone in 9.6 mL aquadest intravenous injection showed a stabile state according to the procedure of the previous method [16], whereas the negative control used was aquadest [17].

2.4. Antibiotic Sensitivity Test

An antibiotic sensitivity test was used in this study that referred to the previous method. Initial preparation was done using forceps to dispense each antimicrobial disk one at a time on nutrient agar that contains cultured bacteria. Agar dilutions in Petri dishes were immediately incubated at 35˚C for 16-18 hours. After that, diameters of inhibition zones were measured in the Petri dishes using a ruler. Results of diameter inhibition zones were compared to the Zone Diameter Interpretive Standard to determine the diameters of the inhibition zones to classify isolates into resistant, intermediate and susceptible depending on the diameters of inhibition zones obtained [18].

2.5. Determination of the Inhibition Zone

The determination of the inhibition zone was conducted according to the previous method. The observation of the inhibition zone was done after the incubation period for 1x24 hours. The inhibition zones around disc papers were measured for four diameter measurements (vertical, horizontal, left diagonal, and right diagonal), and mean inhibition zone was counted by summing up the diameters of the inhibition zones divided by four minus the diameter of the disc paper. The inhibition zone indicated that antibacterial bioactive compounds inhibited the growth of S.typhi and E. Coli in the gonad extracts of *Diadema setosum* [19].

3. Results

Table 1 shows results of chemical screening of the gonad extracts of *Diadema setosum* that contain high antioxidant bioactive compounds as antibacterial agents.

Table 1: Chemical screening of bioactive compounds in the gonad extracts of *Diadema setosum.*

Bioactive Compounds	**Ethyl Acetate Solvent**	**Descriptions**
Alkoloids	-	
Flavonoids	+	Orange to red color
Steroids	+	Greenish color
Saponins	+	Foam for 15 minutes

As shown in Figure 1, the gonad extracts of *Diadema setosum* reduced the growth of *E. coli* at 80% concentration and indicated that this concentration level had a high inhibition response (21 mm in diameter) compared to other concentrations of 40%, 20%, and 10 %.

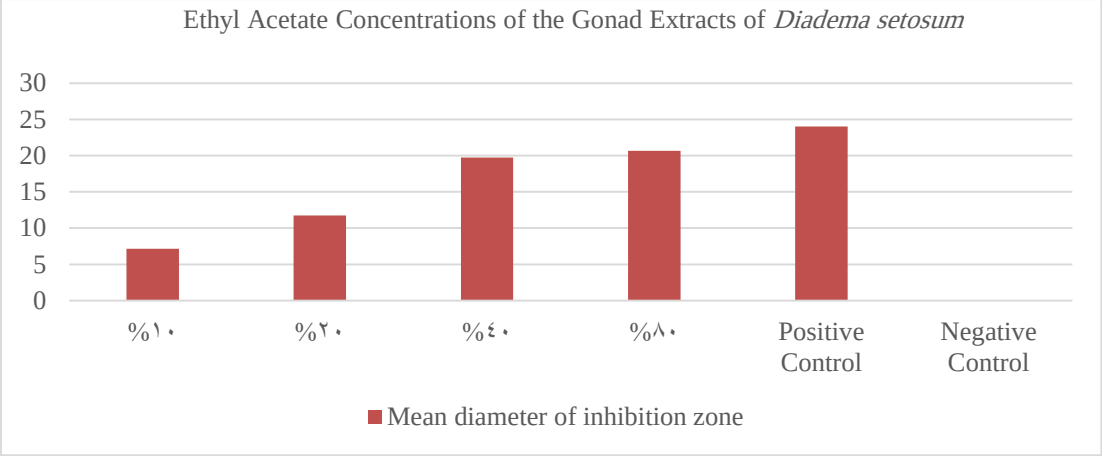

Figure 1: Mean inhibition zone of ethyl acetate extract of the gonad of *Diadema setosum* on the growth of *E. Coli*.

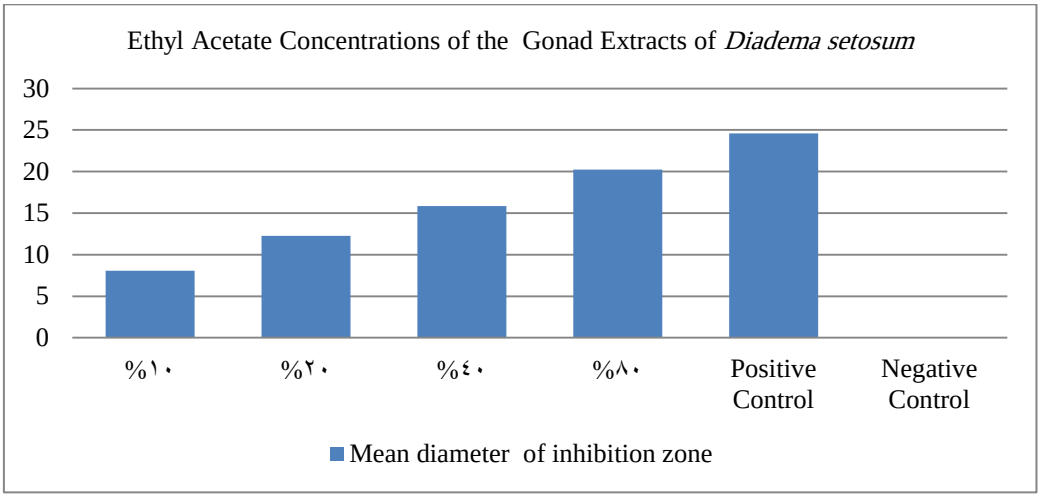

Figure 2: Mean inhibition zone of ethyl acetate extracts of the gonad of *Diadema setosum* on the growth of *S. typhi*.

The gonad extracts of Diadema setosum showed significant inhibition of the growth of S. typhi at 80% concentration with high inhibition response (20 mm in diameter) compared to the concentrations of 40%, 20%, and 10 % with weak inhibition response, as shown in Figure 2.

4. Discussion

Antibiotic is an antibacterial agent derived from microorganisms but not parasites in the form of a synthetic drug used to exterminate or inhibit the growth of other harmful microorganisms to humans. Antibiotics are very toxic that stop bacterial growth and cell division (bacteriostatic) or kill bacteria (bactericide) [20]. However, bacteria can resist antibiotics due to chromosomal mutation or genetic material exchange through transformation, transduction, and conjugation in their plasmids. The overuse of antibiotics or long-term use of antibiotics stimulates these germs more resistance on antibiotics [21]. Concerning this, alternative natural antibacterial

drugs derived from marine resources are necessarily regarded to be used as potential antibacterial agents in minimizing the resistance level of bacteria with minimal side-effects. Several marine species contain bioactive compounds as antibacterial agents and antioxidants such as flavonoids, saponins, and steroids. Our study revealed that the gonad extracts of Diadema setosum derived from the Wakatobi waters of Southeast Province-Indonesia contain bioactive compounds including flavonoids, saponins, and steroids. The three compounds were extracted using ethyl acetate solvent. Our study is consistent with the study of Akerina and his colleagues (2015) that the contents of secondary metabolites of the gonad extracts of Diadema setosum using ethyl acetate solvent (semipolar) contain bioactive compounds of steroids/triterpenoids, saponins and flavonoids [10]. Other studies reported that bioactive compounds including flavonoids and saponins demonstrated their significant levels of inhibition on bacterial growth through inactivation of proteins in the cell membranes through their mechanisms that inhibit permeability of cell membranes of the bacteria that lead to bacterially is by rupture of cell membranes of the bacteria [22]. Steroids have significant roles as an antibacterial agent that associates with their roles to hamper lipid membranes and the sensitivity of bacterial cells by rupture of liposomes leading to bacterial cells to fragile and lysis [23]. Results of our study revealed that microbiological method using the culture of extracts of ethyl acetate of the gonad of Diadema setosum inhibited the growth of E. Coli and S.typhi at 80% concentration with mean inhibition response for E. Coli was 21 mm and mean inhibition response for S.typhi was 20 mm which higher than concentrations of 40%, 20%, and 10 %, as shown in Figure 1 and Figure 2. Our study is consistent with the previous studies that the gonad extracts of Diadema setosum have significant roles as antibacterial compounds, as attested in the study of Salma and his colleagues (2018) derived from a preclinical test of mice BALB/c to be infected by S. typhi. S. type proved the gonad extracts of Diadema setosum (200 mg/kg body weight) reduced the production of antibody IgM and showed the highest increase of IgG antibody titer level was 200 mg/kg ($p=0.004$) at day 7compared to the control group (100 mg/kg body weight) and proved that the gonad extracts of Diadema setosum increased body immune status in patients infected by Salmonella typhi [24].

5. Conclusion

Gonad extracts of *Diadema setosum* contain potential antibacterial agents to be used as a new natural drug by reducing the resistance of *E. coli and S. typhi* at a significant level. In addition, gonads of the *Diadema setosum* contain antioxidant compounds that function as the alternative food in increasing health status and preventing negative effects of bacterial strains of *E. coli and S. typhi.*

Acknowledgements

We would definitely thank Indonesia Ministry of Health and Ministry of Research and Technology, for their financial support (NO:1583^{k}/UN29.20/PPM/2019). Finally, we thank YBLI divers for sample collection.

References

[1] S. R. Lakawa, W. O. Salma, Y. Purnamasari , Asriati, Irawaty, A. Kusnan, et al, “Detection of Salmonella typhi in Urine of 6-12 Years Old Children in The Coastal Region of Southeast Sulawesi Using The

Polymerase Chain Reaction Method," International Journal of Science: Basic and Applied Research (IJSBAR), vol. 43, no.1, pp.175-182, 2019.

[2] L. K. McLellan, A. D. Hunstad, "Urinary Tract Infection: Pathogenesis and Outlook," Trends in Molecular Medicine, vol. 22, no.11, pp. 946–957, 2016.

[3] World Health Organization, "Typhoid Fever," https://www.who.int/features/qa/typhoid-fever/en/. [Sept.13, 2019].

[4] K.O. Akinyemi, A.O.B. Oyefolu, W.B. Mutiu, B.A. Iwalokun, E.S. Ayeni, S.O. Ajose, et al, "Typhoid Fever : Tracking The Trend in Nigeria," The American Journal of Tropical Medicine and Hygiene, vol. 99, no.3, pp. 41-47, 2018.

[5] A.E. Barber, J.P. Norton, T.J. Wiles, M.A. Mulvey, "Strengths and Limitations of Model Systems for the Study of Urinary Tract Infections and Related Pathologies," Microbiology and molecular biology reviews: MMBR, vol.80, no.2, pp.351-67, 2016.

[6] Lindayanti, Muzahar, A.R. Lubis, "Pola resistensi antimikroba pada infeksi saluran kemih yang disebabkan oleh bakteri penghasil ESBL dan non-ESBL," Majalah Kedokteran Nusantara, vol.47, no.2, pp.77-81, 2014.

[7] N.P.H. Lugito, Cucunawangsih, "Antimicrobial Resistance of Salmonella enterica Serovars Typhi and Paratyphi Isolates from a General Hospital in Karawaci, Tangerang, Indonesia: A Five-Year Review," Research Article International Journal of Microbiology, 2017.

[8] I. Indrawati, T.R. Hidayat, N. Rossiana, "Aktivitas Antibakteri dari Bulu Babi (Diadema setosum) Terhadap Escherichia coli dan Staphylococcus aureus," Jurnal Biodjati, vol.3, no.2, pp. 183-192, 2018.

[9] W.O. Salma, S. Wahyuni, N. Kadir, S. As'ad, I. Yusuf, "Effect of Sea Urchin (Diadema setosum) Gonad Extracts on Gene Expression of FOXP3 and The Production of Cytokine on Salmonella typhi- Induced mice," Journal of Applied Pharmaceutical Science, vol.8, no.12, pp.140-146, 2018.

[10]F.O. Akerina, T. Nurhayati, R. Suwandy, "Isolasi dan Karakterisasi Senyawa Antibakteri dari Bulu Babi," Jurnal Pengolahan Hasil Perikanan Indonesia, vol.18, no.1, pp.61-73, 2015.

[11]M. Evi, A.H. Alimuddin, L. Destiarti, "Pemanfaatan Ekstrak Landak Laut (Diadema setosum) dari Pulau Lemukutan Sebagai Antijamur Candida albicans," Jurnal Kimia Khatulistiwa, vol.4, no.4, pp.61-65, 2015.

[12] F. Handayani, R. Sundu, R.M. Sari, "Formulasi Dan Uji Aktivitas Antibakteri Streptococcus Mutans Dari Sediaan Mouthwash Ekstrak Daun Jambu Biji (Psidium Guajava L.)," Jurnal Sains Dan Kesehatan, vol.1, no.8, 2017.

[13] M.R, Tandah, "Daya Hambat Dekokta Kulit Buah Manggis (Garcinia Mangostana L.) Terhadap Bakteri Escherichia Coli," Jurnal Kesehatan Tadulako, vol.2, no.1, pp.1-5, 2016.

[14]M.C. Muntiaha, P. Yamlean, W.A. Lolo, "Uji Efektivitas Sediaan Krim Getah Jarak Cina (Jatropha multifida L.) Untuk Pengobatan Luka Sayat yang Terinfeksi Bakteri Staphylococcus aureus Pada Kelinci (Orytolagus cuniculus)," Jurnal Ilmiah Farmasi Pharmacon Unsran, vol.3, no.3, 2014.

[15]M. Benigna, "Uji Daya Hambat Ekstrak Daun Keji Beling (Srobilanthes crispa BI.) Terhadap Pertumbuhan Bakteri Salmonella typhi Secara In Vitro," Skripsi, Fakultas Keguruan dan Ilmu Pendidikan Universitas Sanata Dharma Yogyakarta ,2015.

[16]Rochepin, "Drug Interaction Center : Seftriakson Intravenous," http://www.rxlist.com/rocephin-drug.html, [Aug.20, 2019].

[17] Mukhriani, "Ekstraksi, Pemisahan Senyawa, dan Identifikasi Senyawa Aktif," Jurnal Kesehatan, vol.7, no.2,2014.

[18] Kuswiyanto, "Bakteriologi 1:Buku Ajar Analis Kesehatan, Jakarta: EGC, 2015.

[19]I.P.W.P. Suherman, "Uji Aktivitas Ekstrak Etanol Daun Dadap Serep (Erythrina lithosperma) Sebagai Antibakteri Terhadap Salmonella typhi," Skripsi, Fakultas Kedokteran Universitas Halu Oleo Kendari, 2016.

[20]A. Syarif, "Farmakologi dan Terapi. Edisi 5. Cetak Ulang Dengan Tambahan," Jakarta: Balai Penerbit FKUI, 2012.

[21]R.F. Jonis, "Pola Resistensi Terhadap Antibiotik Pada Bakteri Salmonella typhi yang diisolasi dari Kultur Darah Pasien Anak Demam Tifoid," Skripsi, Program Studi Pendidikan Dokter Fakultas Kedokteran Universitas Lampung Bandar Lampung, 2018.

[22]R. Yanuarisa, O. Agustina, A. Santoso, "Aktivitas Antibakteri Ekstrak Etanol Daun Tempuyung (Sonchus arvensis L.) Terhadap Salmonella typhi Secara In Vitro," Journal of Agromedicine and Medical Sciences, vol. 2, no.2, 2016.

[23]A.B. Shinde, Y.R Mulay, "Phytochemical Analysis and Antibacterial Properties of Some Selected Indian Medicinal Plants," International Journal of Current Microbiology and Applied Science, vol.4, no.3, pp.228-235, 2015.

[24]W.O. Salma, I. Yusuf, M. Karo, L. Banudi, "The effect of Sea urchin (Diadema setosum) gonad extract on IgM and IgG antibodies production in BALB/c mice infected by Salmonella Typhi," The Indonesian Journal Of Clinical Nutrition (IJCN), vol.14, no.3, pp.93-98, 2018.