ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antibacterial Activity of Fingerroot (*Boesenbergia rotunda*) Extract against Acne-Inducing Bacteria.

Mohd Azuar Hamizan Rahman[1], Lau Kah Yan[1], and Yaya Rukayadi[1,2]*.

[1]Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.
[2]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.
[1,2]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.

ABSTRACT

Fingerroot (Boesenbergia rotunda) is a medicinal and culinary herb in the ginger family (Zingiberaceae). It has been used for beauty and skin treatment, however, antibacterial activity of B. rotunda extract against acne-causing bacteria has not been investigated. The aim of this study is to analyse the antibacterial activity of B. rotunda extract acne-inducing bacteria; Propionibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis. The methanolic extract of B. rotunda was tested for antimicrobial activity against acne-causing bacteria in term of disk diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and kill-time curve using the standard methods of Clinical and Laboratory Standard Institute (CLSI). The B. rotunda extract has inhibited the growth of P. acnes ATCC6919, P. acnes clinical strain, S. epidermidis KCCM40003 and S. aureus KCCM12255 with MIC value of 0.63-, 0.63-, 0.31-, and 0.02 mg/mL, respectively. Moreover, MBC of B. rotunda extract was 1.25-, 1.25-, 0.63-, and 0.04 mg/mL on P. acnes ATCC6919, P. acnes clinical strain, S. epidermidis KCCM40003 and S. aureus KCCM12255, respectively. Time–kill curves analyses demonstrated that the bactericidal endpoint of B. rotunda extract on P. acnes ATCC6919, P. acnes clinical strain, and S. aureus KCCM12255 was 4× MIC after 4 h of incubation. Meanwhile, endpoint of B. rotunda extract on S. epidermidis was killed at 8×MIC after 0.5 h of incubation. The result show that B. rotunda extract have potent antibacterial activity against acne-inducing bacteria.
Keywords: antibacterial, acne, fingerroot, *Boesenbergia rotunda, temukunci.*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Acne is the most common dermatological disease among the general population, and is characterized by comedones (black and whiteheads), inflammatory lesions, secondary depigmentation and scarring [1]. There are several acne-causing factors including increased sebum production, ductal cornification, abnormal bacterial colonization and inflammation [2-4]. The sebum hyper secretion also promotes the growth of anaerobic Propionibacterium acnes [5]. In addition, the yeast of Malassezia spp. or Pitryosporum ovale, Staphylococcus aureus and Staphylococcus epidermidis are also found in the skin of patient with inflammatory acne [2, 6, 7]. Excessive inflammation in the subsequent healing tissue is conducive to scarring [8], affects a significant number of adolescents with regard to the impaired physical appearance, decreased self-esteem [5], feelings of inferiority as well as insecurity, and at worst, a permanent physical scar [9].

Topical application of therapeutic agents has been found to be more effective in acne treatment than hormonal treatment and laser therapy [5]. However, the widespread use of antibiotics as the therapeutic agents has led to the development of drug resistant strains [10, 3, 11]. The emergence of bacterial resistance prompted an extensive research to find novel antimicrobial compounds or therapeutic alternatives [11, 12]. Thus, naturally derived compounds from herbs or medicinal plants have been targeted as source of natural antibacterial agent as they have lesser adverse effects than synthetic agents [5]. Previously unexplored traditional herbal medicines provide a great potential for the development of new skin-care products [13].

One of the traditional medicinal plants of interest is Boesenbergia rotunda (Zingiberaceae), also known as fingerroot, temu kunci in Indonesia or krachai in Thailand. It has been used traditionally for food and medicinal purposes, such as cure diarrhea. B. rotunda also scientifically reported to contain antimutagenic, antitumor, and anti-inflammatory activities [14]. Isopanduratin A derived from B. rotunda was a potential antibacterial agent against cariogenic Streptococcus mutans [14], while panduratin A demonstrates in vitro antistaphylococcal activities [15]. This led to the suggestion that B. rotunda may exhibit antibacterial activity against acne-causing bacteria; Propionibacterium acnes, Staphylococcus aureus and S. epidermidis.

In this study the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the B. rotunda have been determined using Clinical and Laboratory Standard Institute (CLSI) methods. Time–kill curves also have been evaluated to assess the correlation between MIC and MBC activity of B. rotunda extract at different concentrations, ranging from 0×MIC to 8×MIC.

MATERIALS AND METHODS

PLANT MATERIALS:

The dried rhizome of B. rotunda was purchased from traditional herbal market in Bandung, Indonesia and identified by Institute of Bioscience, Universiti Putra Malaysia (Selangor, Malaysia). The voucher specimen is deposited in the Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia.

PLANT EXTRACT PREPARATION:

The dried rhizome of *B. rotunda* (100 g) was ground and extracted with 400 ml of 100% (v/v) methanol for 1 week at room temperature [16], with slight modification. The plant extract was filtered with Whatman filter paper No.1 (Whatman International Ltd., Middlesex, England) and concentrated with a rotary vacuum evaporator (Heidolph VV2011, Schwabach, Germany) at 50 ˚C, yielding methanolic extract. The methanolic extract was dissolved in 10% aqueous dimethylsulfoxide (DMSO) to obtain 100 mg/mL and the solution was further diluted in 1:10 (v/v) DMSO to obtain 10 mg/mL stock solutions. A 10% DMSO was found not to kill the bacteria tested in this research.

TESTED MICROORGANISMS AND INOCULUM PREPARATION:

Propionibacterium *acnes* ATCC6919 and P. acnes clinical strain was obtained from the American Type Culture Collection (Rockville, MD, USA) and Laboratory of Natural Products (LHS), division of Microbiology,

respectively. Staphylococcus epidermidis KCCM40003 and S. aureus KCCM12255 were obtained from Korean Culture Center of Microorganisms (Seoul, South Korea).

P. acnes, S. epidermidis and S. aureus were grown on Mueller Hinton agar (MHA) (Difco, Franklin Lakes, NJ, USA), aerobically for 24 hours at 37˚C prior to use, whereas inoculum cell suspension was prepared by propagating a single colony of each bacterial species in 10 mL of Mueller Hinton broth (MHB) at 37˚C overnight with 200 rpm agitation. A 1 µL of bacteria suspension was further diluted 1:10 in MHB to yield inoculum with ~10^7 – 10^8 CFU/mL prior to use.

DISK DIFFUSION TEST:

B. rotunda extract was tested for antimicrobial activity using the standard paper disk diffusion assay [17]. Each bacterial species was streaked on MHA plates with a sterile cotton swab. Sterile filter paper discs, 6 mm diameter, were placed on top and 10 µL of 10 mg/mL (w/v) *B. rotunda* extract was loaded on the paper discs. A 1 mg/mL of chlorhexidine (CHX) was used as positive control in the assay. The plates were incubated at 37˚C for 12-24 hours. Evidence of clear zone indicates bacterial growth inhibition and was measured in mm.

MIC AND MBC DETERMINATION:

In vitro susceptibility tests were performed in a 96-well microtiter plate to determine MIC and MBC of B. rotunda extract against P. acnes ATCC6919, P. acnes clinical strain, S. epidermidis KCCM40003 and S. aureus KCCM12255 using standard broth microdilution methods with an inoculum ~10^7 – 10^8 CFU/mL [17]. Briefly, a 2-fold B. rotunda extract stock solution was mixed with the test organisms in MHB. Column 12 of the microtiter plate contained the highest concentration of the extract, while column 3 contained the lowest concentration. Column 2 served as the positive control for all samples (only MHB and inoculums), and column 1 as the negative control (only MHB, no inoculum and antimicrobial agent). Microtiter plates were incubated aerobically at 37˚C for 24 hours. The MIC was defined as the lowest concentration of antimicrobial agent that resulted in the inhibition of visible growth.

MBCs were determined for each bacterial species as outlined for MIC by taking 10 µL of the media from each well showing no visible growth and sub-culturing onto MHA plates [15]. The plates were incubated at 37˚C for 24 hours until growth was seen in the growth control plates. MBC was defined as the corresponding concentrations required killing the microorganisms completely.

TIME–KILL ASSAY:

Time–kill assay was performed on each bacterial species in MHB medium [16, 17], with modification. Briefly, the inoculum suspension of P. acnes, S. epidermidis and S. aureus was approximately 10^7 – 10^8 CFU/mL. The B. rotunda extract was diluted with the MHB medium containing inoculum to obtain final concentrations of 0×MIC, 0.5×MIC, 1×MIC, 2×MIC, 4×MIC, and 8×MIC for each bacterial species. Cultures (1 ml final volume) were incubated at 37˚C with 200 rpm agitation. At pre-determined time points (0, 0.5, 1, 2, and 4 hours), 100 µL aliquots were removed and transferred to Eppendorf tubes. The aliquot was serially diluted with 990 µL of 1% phosphate buffered saline (PBS) and 20 µL was spread onto MHA plates and incubated at 37˚C for 24 hours. The number of colonies appeared on the plates was counted, to determine the number of CFU/mL. Assays were carried out in triplicates.

RESULTS AND DISCUSSION

The antimicrobial activity of B. rotunda extract against P. acnes ATCC6919, P. acnes clinical strains, S. epidermidis KCCM40003 and S. aureus KCCM12255 is summarized in Table 1. Table 1 shows B. rotunda extract with 10 mg/mL showed antibacterial activity against P. acnes ATCC6919, P. acnes clinical strains, S. epidermidis KCCM40003 and S. aureus KCCM12255 with a clear inhibition zone of 10, 9, 8, and 9 mm, respectively. In comparison, inhibition zone of the positive control, chlorhexidine against P. acnes ATCC 6919, P. acnes clinical strain, S. epidermidis KCCM40003 and S. aureus KCCM12255 were 18, 18, 14, and 12 mm, respectively. The MICs of the B. rotunda extract against P. acnes ATCC6919 and P. acnes clinical strain was 0.63 mg/mL, followed by S. epidermidis KCCM40003, 0.31 mg/mL, and S. aureus KCCM12255, 0.02 mg/mL. Lower MICs value

denotes greater antibacterial activity of the extract. While the MBCs of the B. rotunda extract against P. acnes ATCC6919, P. acnes clinical strain, S. epidermidis KCCM40003, and S. aureus KCCM12255 were 1.25, 1.25, 0.63, and 0.04 mg/mL, respectively.

Table 1. Inhibition zone, minimum inhibitory concentration, and minimum bactericidal concentration of *B. rotunda* extract against acne-inducing bacteria

Bacterial strains	Inhibition Zone (mm)	MIC (mg/mL)	MBC (mg/mL)
Propionibacterium acnes			
ATCC 6919	10	0.63	1.25
Clinical	9	0.63	1.25
Staphylococcus epidermidis	8	0.31	0.63
Staphylococcus aureus	9	0.02	0.04

Disk diffusion test served as a preliminary check for antibacterial activity [18], as the diameters of the inhibition zone did not always linearly correlate to the MICs of plant extracts [19]. For instance, both the clinical strain of P. acnes and S. aureus has a 9 mm inhibition zone but MICs value was 0.63, and 0.02 mg/mL, respectively. Moreover, the diameters of the inhibition zones are dependent on the active compounds solubility and rate of diffusion through an agar medium [20, 19]. Moreover, the hydrophobic properties of certain plant extracts can affect the uniformity of diffusion through the media [19]. Thus, the antibacterial activity of plant extract may be more accurately evaluated using MIC values [20, 19].

Some plant extracts exhibited higher antibacterial effects against anaerobic bacteria than against aerobic bacteria [11]. However, the same cannot be applied for B. rotunda extract. P. acnes, a facultative anaerobic bacterium, was found to have higher MIC value, 0.63 mg/mL, whereas lower MIC value were observed for the aerobic bacteria, S. epidermidis and S. aureus, 0.31 mg/ml and 0.02 mg/mL, respectively. P. acnes, S. epidermidis and S. aureus are all Gram-positive bacteria. In accordance with previous findings, Gram-positive bacteria were more susceptible to plant extracts compared to Gram-negative bacteria due to the presence of lipopolysaccharides in outer membrane of the latter [21].

The MICs value of B. rotunda extract against P. acnes (0.63 mg/mL) was weaker than those of Garcinia mangostana (0.039 mg/mL), Hautuynia cordata (0.039 mg/mL) and Coscinium fenestratum (0.049 mg/mL) [10, 22]. However, The MICs value of B. rotunda extract against P. acnes (0.63 mg/mL) was equal with Andrographis paniculata (0.625 mg/mL), Eupatorium odoratum (0.625 mg/mL), Senna alata (0.625 mg/mL) [10]. Interestingly, the MICs value of B. rotunda extract was stronger than that of the MICs value of Azadirachta indica (5 mg/mL), Barleria lupulina (1.25 mg/mL), Carthamus tinctorius (>5 mg/mL), Centella asiatica (5 mg/mL), Clinacanthus nutans (>5 mg/mL), Cymbopogon citratus (5 mg/mL), Hibiscus sabdariffa (2.5 mg/mL), Lawsonia inermis (2.5 mg/mL), Lycopersicon esculentum (>5 mg/mL), Murdannia loriformis (>5 mg/mL), Psidium guajava (2.5 mg/mL), Senna occidentalis (2.5 mg/mL), Senna siamea (1.25 mg/mL) and Tagetes erecta (2.5 mg/mL) [10].

MIC value of B. rotunda against S. epidermidis was 0.31 mg/mL, meaning B. rotunda extract was stronger than those of Andrographis paniculata, Eupatorium odoratum, Senna alata, Azadirachta indica, Barleria lupulina, Carthamus tinctorius, Centella asiatica, Clinacanthus nutans, Cymbopogon citratus, Hibiscus sabdariffa, Lawsonia inermis, Lycopersicon esculentum, Murdannia loriformis, Psidium guajava, Senna occidentalis and Tagetes erecta [10]. Nevertheless, the MIC value of B. rotunda extract was weaker than those of Garcinia mangostana, Hautuynia cordata and Coscinium fenestratum [10,22]. While, the MIC value of B. rotunda against S. aureus (0.02 mg/mL) compared with other extracts such as the extract of Salvia officinalis, Eucalyptus globulus, Coleus forskohlii, Coptis chinensis, Turnera diffusa, and Larrea tridentata exhibited MIC values ranging from 0.06 to 0.3 mg/mL [23]. Thus, the MIC value of B. rotunda against S. aureus was stronger than those of Salvia officinalis, Eucalyptus globulus, Coleus forskohlii, Coptis chinensis, Turnera diffusa, and Larrea tridentate.

ISSN: 0975-8585

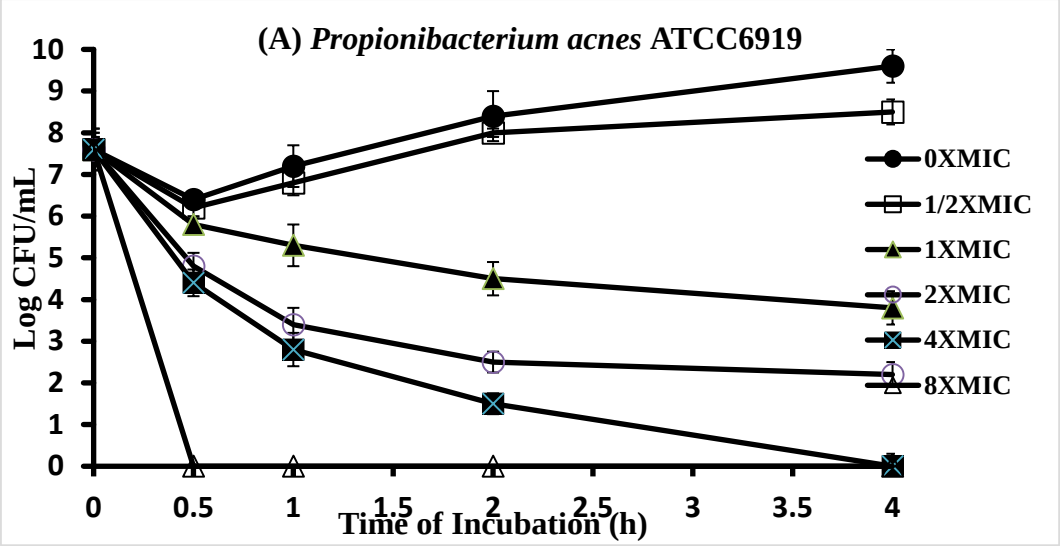

Figure 1 (A)

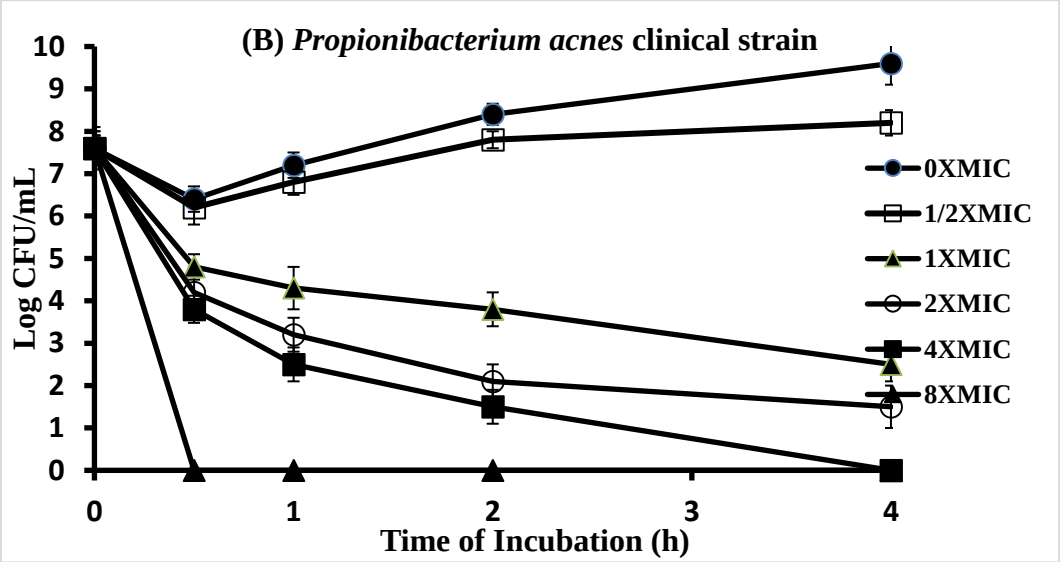

Figure 1 (B)

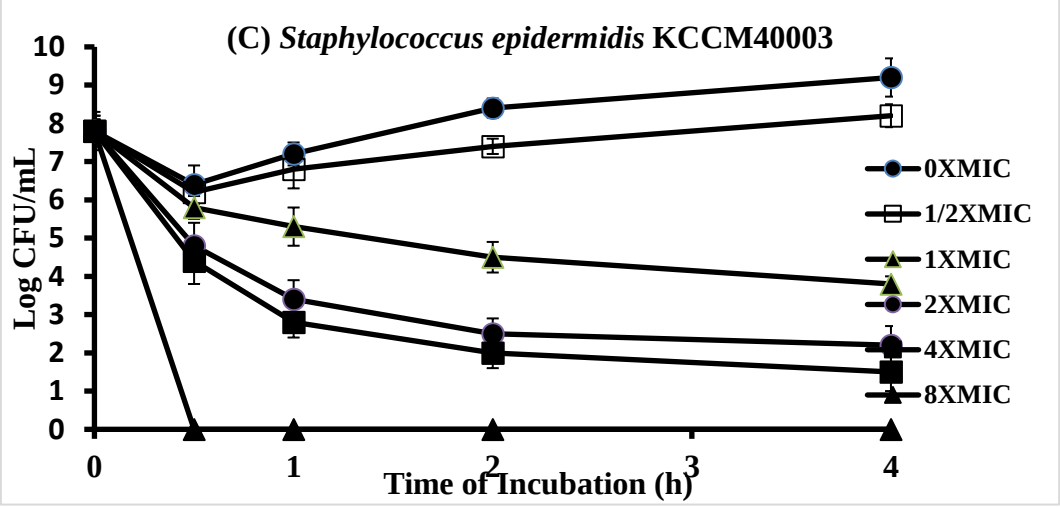

Figure 1 (C)

ISSN: 0975-8585

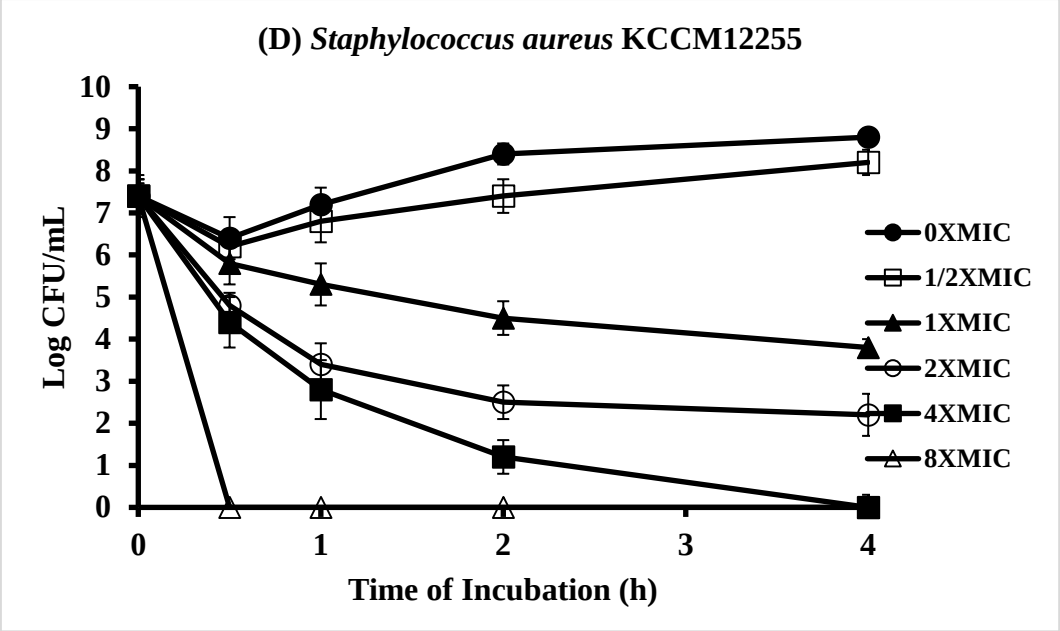

Figure (D)

Figure 1. Representative time–kill plots for acne-causing bacteria species following exposure to *B. rotunda* extract at 0×MIC (filled circles), 0.5×MIC (open squares), 1× MIC (filled triangles), 2×MIC (open circles), 4×MIC (filled squares), and 8×MIC (open triangles) after endpoint (4 h). (A) *P. acnes* ATCC6919 (0, 313, 625, 1250, 2500 and 5000 mg/mL); (B) *P. acnes* Clinical strain (0, 313, 625, 1250, 2500 and 5000 mg/mL); (C) *S. epidermidis* KCCM40003 (0, 156, 313, 625, 1250 and 2500 mg/mL); and (D) *S. aureus* KCCM12255 (0, 10, 20, 40, 80 and 160 mg/mL). Values in the brackets after each species denote 0×MIC, 0.5×MIC, 1×MIC, 2×MIC, 4×MIC and 8×MIC, respectively.

The time-kill curves of B. rotunda extract against the acne-causing bacteria species is presented in Figure 1 (A, B, C, D). The time-kill curve of B. rotunda extract on P. acnes ATCC6919, P. acnes clinical strains, S. epidermidis KCCM40003 and S. aureus KCCM12255 was fast acting; the reduction of bacteria was ≥3 log CFU/mL at 2× MIC after 1 h (Figure 1). The complete killing of B. rotunda extract against P. acnes ATCC6919 (Figure 1A), P. acnes clinical strain (Figure 1B), and S. aureus KCCM12255 (Figure 1D) was reached at concentration of 4×MIC after 4 h of incubation at concentration of 8×MIC after 0.5 h of incubation. Meanwhile, S. epidermidis KCCM40003 could completely killed by B. rotunda extract at concentration of 8×MIC after 0.5 h of incubation (Figure 1C). These data demonstrated that the bactericidal ability of B. rotunda extract is dependent on the concentration and bacterial species. The antibacterial activity is most likely due to the adsorption of phytochemical constituents causing membrane disruption, subsequent leakage of cellular contents and cell death [24, 25]. Overall, B. rotunda extract shows strong antimicrobial activity against acne-inducing bacteria. Further study on the active constituents and other possible advantages of B. rotunda extract, such as antioxidant and anti-inflammatory activity would be interesting. The research and development of natural plant extracts would be beneficial to the future growth cosmeceuticals and nutraceuticals.

CONCLUSION

Boesenbergia rotunda extract shows strong antimicrobial activity against acne-inducing bacteria; Propionibacterium acnes, Staphylococcus epidermidis and S. aureus. Thus, B. rotunda extract can be developed as natural anti-acne agent.

ACKNOWLEDGEMENT

The authors would like to acknowledge financial support from GP-IPS/2015/960300 Grant Putra UPM, Malaysia to Yaya Rukayadi.

ISSN: 0975-8585

REFERENCES

[1] Perkins AC, Cheng CE, Hillebrand GG, Miyamoto K, Kimball A. J. Eur. Dermato. Ven. 2011; 25(9): 1054-1060.
[2] Jappe U. Acta Dermato-Venereologica 2003; 83(4): 241-248.
[3] Viyoch J, Pisutthanan N, Faikreua A, Nupangta K, Wangtorpol K, Ngokkuen J. Int. J. Cosmet. Sci. 2006; 28(2):125-133.
[4] Tahir CM. J. Pakistan Ass. Dermatol. 2010; 20(2): 93-97.
[5] Kanlayavattanakul M, Lourith N. Int. J. Cosmet. Scie. 2011; 33(4): 289-297.
[6] Grange PA, Weill B, Dupin N, Batteux F. Microbes and Infection 2010; 12 (14-15): 1085-1090.
[7] Khorvash F, Abdi F, Kashani HH, Naeini FF, Narimani T. North Amer. J. Med. Sci. 2012; 4(11): 573-576.
[8] Holland DB, Jeremy AH, Roberts SG, Seukeran DC, Layton AM, Cunliffe WJ. J. Dermatol. 2004; 150(1): 72-81.
[9] Rivera AE. J. Amer. Acad. Dermatol. 2008; 59(4): 659-676.
[10] Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W. J. Ethnopharmacol. 2005; 101(1–3): 330-333.
[11] Choi JS, Bae HJ, Kim SJ, Choi IS. J. Environ. Biol. 2011; 2(3): 313-318.
[12] Katsambas A, Dessinioti C. Dermatol. Theraphy 2008; 21(2): 86-95.
[13] Kim SS, Hyun CG, Kim JY, Lee J, Park D. J. App. Biol. Chem. 2007; 50(2): 101-103.
[14] Hwang JK, Chung JY, Baek NI, Park JH. Int. J. Antimicrobiol. Agent 2004; 23(4): 377-381.
[15] Rukayadi Y, Lee KH, Han S, Yong D, Hwang JK. Antimicrob. Agents Chemother. 2009; 3(10): 4529-4532.
[16] Rukayadi Y, Yong D, Hwang JK. J. Antimicrob. Chemother. 2006; 7(6): 1231-1234.
[17] Wikler MA. Clinical and Laboratory Standards Institute. 2009. Method for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically 2012; Approved Standard M07-A8.
[18] Burt S. Int. J. Food Microbiol. 2004; 94(3): 223-253.
[19] Jun H, Kim J, Bang J, Kim H, Beuchat LR, Ryu JH. Int. J. Food Microbiol. 2013; 160(3): 260-266.
[20] Aqil F, Ahmad I, Owais M. Biotechnol. J. 2006; 1(10): 1093-1102.
[21] Alzoreky NS, Nakahara K. Int. J. Food Microbiol. 2003; 80(3): 223-230.
[22] Kumar GS, Jayaveera KN, Ashok Kumar CK, Sanjay UP, Swamy BMW, Kumar DVK. Tropical J. Pharm. Res. 20017; 6 (2):717-723.
[23] Snowden R, Harrington H, Morrill K, Jeane L, … Langland J. The Journal of Alternative and Complementary Medicine 2014; 20 (5):375-382.
[24] Fook YC, Kheng YS. Int. J. Pharmacol. 2009; 5(5): 285-297.
[25] Negi PS. Int. J. Food Microbiol. 2012; 156(1), 7-17.