Antibacterial activity of extracts and compounds from the roots of *Sesbania grandiflora* (Leguminosae)

Noviany[1*], Osman Hasnah[2], Mohamad Suriyati[3] and Hadi Sutopo[1]
1. Department of Chemistry, Faculty of Matematics and Natural Sciences, University of Lampung, 35145, Bandar Lampung, INDONESIA
2. School of Chemical Sciences, Universiti Sains Malaysia, Minden 11800, Penang, MALAYSIA
3. School of Biological Sciences, Universiti Sains Malaysia, Minden 11800, Penang, MALAYSIA
*noviany@fmipa.unila.ac.id

Abstract
Four compounds betulinic acid, 7,4'-dihydroxy-2'-methoxyisoflavan, 3-hydroxy-9-methoxypterocarpan and 7-hydroxy-2',4'-dimethoxyisoflavan were separated from the roots of Sesbania grandiflora. Structure elucidation of the compounds was determined using UV, IR, MS and NMR spectroscopic techniques. Root extracts and purified compounds were screened for the antibacterial activity by disc-diffusion method against nine bacterial. The activity results of the test components were shown to be dose dependent.

The methanol extract displayed the growth inhibition of Streptococcus pyogenes, Bacillus cereus, Staphyloccus aureus and Klebsiella pneumoniae. The acetone extract inhibited the growth of S. pyogenes, B. cereus, S. aureus, Streptococcus pneumoniae, Haemophilus influenzae and K. pneumoniae. The results partially justify the use of S. grandiflora root in traditional medicine to treat diseases caused by bacterial infection.

Keywords: *Sesbania grandiflora*, Antibacterial activity, betulinic acid, 7,4'-dihydroxy-2'-methoxyisoflavan, 3-hydroxy-9-methoxypterocarpan, 7-hydroxy-2',4'-dimethoxyisoflavan.

Introduction
Leguminosae, one large group of plants comprising of about 20,000 species, are classified into around 727 genera[1]. It is widely distributed throughout the world. Many species of the Leguminosae family are frequently used in many cultures worldwide as traditional herbal medicine in the treatment of various illnesses such as diabetes, cough, urinary disease, eye disease, lung disease, stomach ache, toothache, fever, dysentery and various bacterial infections including inflammation of the skin and mucous membranes[2-6].

Many studies were undertaken to test many genera of this family against different types of cancer cells including breast cancer, human endometrial cell line and human leukemia HL60 cells[7-12]. In view of their long-standing use as traditional remedy for curing diseases, further investigations are needed to verify these plants as potential sources of antimicrobial compounds especially the least studied species including *S. grandiflora* (L.) Pers.

S. grandiflora belongs to the subfamily Faboideae and tribe Robinieae. This plant is widely distributed in tropical dry and moist forests from sea level to 800 m of Asian countries including Indonesia, Malaysia and Philippines. The local name of this plant in Indonesia is turi. The roots, bark, gum, leaves, flowers and fruits of this plant are employed in folk remedy to cure a variety of ailments including microbial infections[13-15]. Several studies have been conducted on various parts of *S. grandiflora* with regards to their phytochemical and pharmacological properties. A number of compounds such as sterols, saponins and tannins which exhibited effects such as hypotensive, oedema and diuretic had been identified from the crude extracts of *S. grandiflora*[16].

A study by Kasture et al[17] demonstrated that the leaf fraction of *S. grandiflora* containing triterpene exhibited anticonvulsant and anxiolytic activities. Another study revealed that the leaves of *S. grandiflora* showed protective effect against erythromycin estolate-induced hepatotoxicity[18]. Recently, supplementation of *S. grandiflora* leaves had been shown to prevent lung oxidative damage and reverse cigarette smoke-induced oxidative damage in rats[19,20]. The methanol and methylene chloride extracts of aerial parts of *S. grandiflora* displayed the antifungal activities[21].

The current research was undertaken to elaborate the antibacterial property of *S. grandiflora* root extract, fraction and purified compounds and verify scientifically their uses in folk medicine for curing diseases caused by bacterial infections.

Material and Methods
General experimental procedures: A digital polarimeter (JASCO, DIP-370) with 0.5 cm microcell was used to determine optical rotation. A Bruker Avance 300 spectrometer, operating at 300 MHz for ^{1}H and ^{13}C at 400 MHz was used for 2D-experiments with Me_4Si as internal standard. Chemical shifts are expressed in ppm (parts per million) referring to the solvent peaks δ H 3.34 and δ C 49.0 for CD_3COCD_3, δ H 7.27 and δ C 77.0 for $CDCl_3$. Coupling constant J is expressed in hertz. A Micro TOF-Q MS (positive-ion mode) was used to measure HRESIMS spectra. A Perkin-Elmer system 2000 FT-IR Spectrometer was applied to record the IR spectra using potassium bromide disc procedure. The range of measurement was from 4000 to 650 cm^{-1}.

A Perkin-Elmer Lambda 25 Spectrometer was used to record UV spectra. TLC was performed on pre-coated Merck plastic sheets (silica gel 60 PF254, 0.25 mm) and the plates were sprayed with $Ce(SO_4)_2.H_2O$ development. Kieselgel 60 of silica gel (230-400 mesh ASTM) was used to conduct column chromatography.

Plant materials: *S. grandiflora* roots were assembled in Sidosari district, Lampung, Indonesia. Identification of plant was done by Dr. Harry Wiriadinata and the specimens (NV1/NRGD/2008) were deposited at the Herbarium Bogoriense, LIPI Bogor, Indonesia.

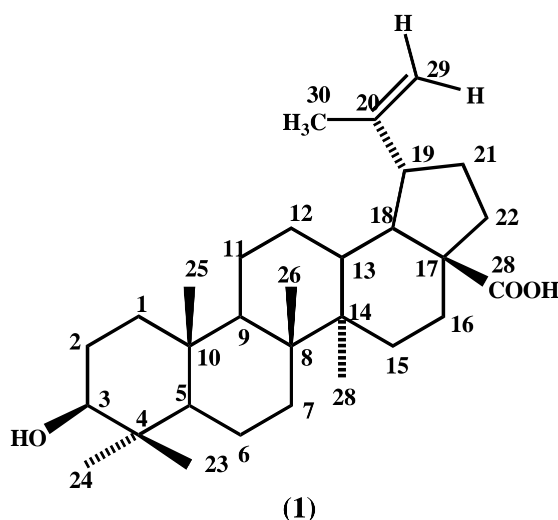
Extraction and isolation: The experiment was done following the method that had been described in previous study[22]. Briefly, 1.5 kg of powdered roots of sampel were macerated using methanol (90%) for three times at room temperature to yield methanol (MeOH) extract. Compound 1 was obtained by recrystalisation from methanol extract while compound 2-4 were afforded by repeated centrifugal TLC. The crude MeOH extract was fractionated by partition technique using *n*-hexane and MeOH sequentially to yield hexane-soluble and MeOH-soluble fraction respectively. The structure elucidation of pure constituents 1–4 was assessed by spectroscopy analyses (NMR and MS) and by comparison of their physical properties as well as the spectroscopic data with those reported earlier. Compounds 1, 2, 3 and 4 were identified as betulinic acid; 7,4'-dihydroxy-2'-methoxyisoflavan; 3-hydroxy-9-methoxypterocarpan and 7-hydroxy-2',4'-dimethoxyisoflavan respectively (Figure 1)[22].

Antimicrobial activity

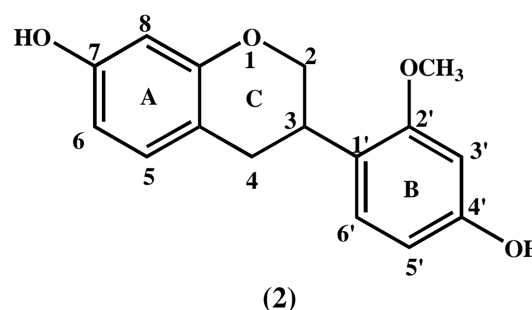
Test microorganisms: Nine aerobic bacterial strains, four gram-positive: *Streptococcus pyogenes* ATCC 19615, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 6303 and five gram-negative *Haemophilus influenzae* ATCC 10211, *Salmonella typhimurium* ATCC 13311, *Shigella flexneri* ATCC 12022, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883 were used for the antibacterial activity assay. The bacteria were cultured and maintained on nutrient agar slant at 4°C. Prior to use, each bacterial strain was activated by transferring a loopful from the stock culture onto NA and then incubated at 37°C for 24 hours.

Antibacterial activity test: The antibacterial activity assay was performed using the disc diffusion method[23]. Bacterial inoculum was prepared by inoculating a loopful of each activated bacterial strain into a tube of sterile saline solution. The turbidity of the inoculum was adjusted to match the McFarland 0.5 solution. Each inoculum was evenly swabbed onto a 15 cm diameter plate of Mueller Hinton agar surface by using a sterile cotton swab. The test components (extract/fraction and compounds) were dissolved in dimethyl sulfoxide (DMSO) and sterilised by filtration using 0.22 µm cellulose membrane filter.

Each component was further diluted in the same solvent to give different concentrations of 0.3, 0.6, 1.2, 2, 4, 8 mg/mL (extract/fraction) and 0.025, 0.05, 0.1, 0.2, 0.4, 1 mg/mL (compounds). Sterile blank discs (6 mm) were aseptically impregnated with 20 µL of each test component and then deposited on the surface of the inoculated plate giving final test doses of 6, 12, 24, 40, 80, 160 µg/disc (extract/fraction) and 0.5, 1, 2, 4, 8, 20 µg/disc (compounds). The tests were carried out in duplicate.



(1)



(2)

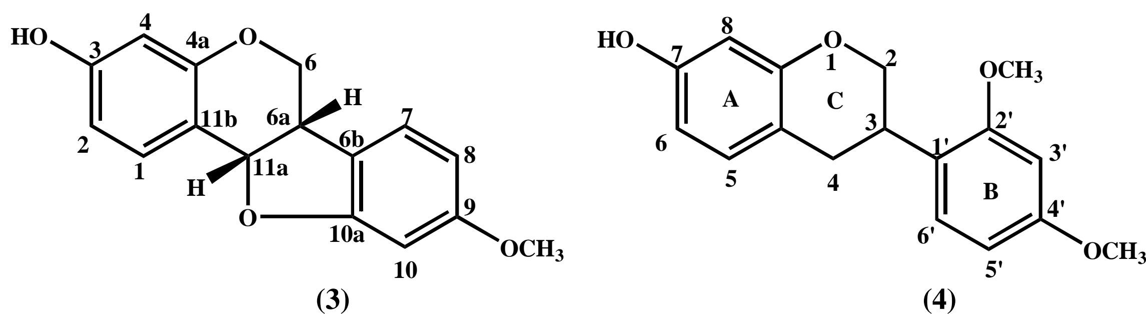


Figure 1: The compounds isolated from *S. grandiflora* root

In parallel, a control test was performed individually against each test microorganism using DMSO as a negative control and commercially prepared antibiotic discs (6 mm) as standard positive controls. The antibiotics were tetracycline (30 µg/disc), gentamicin (10 µg/disc), cefuroxime (30 µg/disc) and amoxicillin (25 µg/disc). The plates were incubated at 37°C for 24 hours. The antibacterial activity was established by the presence of clear zone of inhibition (no growth) around the test disc and assessed based on the measurement of the inhibition zone.

Results and Discussion

In the present study, the antibacterial property of methanol extract and acetone fraction and purified compounds from the roots of *S. grandiflora* collected from Sidosari, Indonesia, were tested against a range of aerobic bacteria. The antibacterial property of *S. grandiflora* extract/fraction and compounds against the test bacteria was quantified by the presence or absence of zones of inhibition and their diameters (Table 1).

The results showed that the antibacterial activity of the test components was dose dependent. The methanol extract inhibited the growth of three of the gram-positive bacteria (*S. pyogenes*, *B. cereus and S. aureus*) and only one of the gram-negative bacteria (*K. pneumoniae*). The most susceptible bacteria to the methanol extract were *B. cereus*. The acetone fraction inhibited the growth of all the gram-positive bacteria and two of the gram-negative bacteria (*H. influenzae* and *K. pneumonia*). *S. aureus* was observed to be the most susceptible to the acetone fraction.

Betulinic acid (1) had no antibacterial activity against all the test bacteria. whereas both 7,4'-dihydroxy-2'-methoxyisoflavan (2) and 3-hydroxy-9-methoxypterocarpan (3) similarly inhibited *S. pyogenes, B. cereus* and *K. pneumoniae*; and 7-hydroxy-2',4'-dimethoxyisoflavan (4) inhibited only the gram-positive bacteria (*S. pyogenes* and *B. cereus*). *S. aureus*, *S. pneumoniae, K. pneumonia* and *E. coli* were not tested against 7-hydroxy-2',4'-dimethoxyisoflavan (4). Among the test bacteria, *S. typhimurium*, *S. flexneri* and *E. coli*, were found to be resistant to all of the test components. In contrast, *S. pyogenes*, *B. cereus* and *K. pneumoniae* were observed to be susceptible to all of the active components. The growth of all the test bacteria was not affected by the negative control DMSO.

The results indicated that gram-positive bacteria were more sensitive to the test components compared to gram-negative bacteria. The difference in susceptibility between these two groups of bacteria could be due to the different structures of the bacterial cell membrane. Gram-negative bacteria possess high lipopolysaccharide content in their outer membrane which causes the cell wall to be impermeable to chemical substances[24]. Gram-positive bacteria possess only an outer peptidoglycan layer which is not an effective permeability barrier.

Furthermore, the cell wall of Gram-negative bacteria is more complex than gram-positive bacteria, therefore, gram-positive bacteria are more susceptible to antimicrobial agents than gram-negative bacteria[25,26].

As expected, *S. grandiflora* root acetone fraction exhibited stronger antibacterial activity than its methanol extract since the methanol extract was slightly more polar than acetone fraction. Previous report had demonstrated that being highly polar, methanol was unable to extract as much of the active compounds compared to non-polar solvents[27].

The results in the present study indicate that the active compounds responsible for antibacterial activity of acetone fraction were relatively higher in concentration. It was also supported that the active compounds 2 was afforded from the acetone fraction while the inactive compound 1 was obtained from the methanol extract.

With regards to the purified compounds, the bacterial growth inhibitions of the active compounds 2, 3 and 4 were not significantly different (7-13 mm at 8-20 µg/disc). The antibacterial activity of these isoflavanoid compounds could be due to the isoflavan skeleton in their parent structures and in addition the pterocarpan skeleton in compound 3. Previous studies have investigated the antimicrobial activities of different molecular structures of isoflavanoids against different strains of microorganisms[28-30].

In these studies, both isoflavan and pterocarpan skeleton were generally found to be active against most of the tested microorganisms. It seemed that the presence of hydroxyl groups at positions C-7 in compounds 2 and 4 and at position C-3 in compound 3 could influence their antimicrobial activity against the tested bacterial strains. The slightly reduced antibacterial activity of compound 3 could probably

be due to the minor change in its ring formation. In this present work, compound 1 (betulinic acid) had no antibacterial activity against all the tested strains. The loss of antibacterial activity could be attributed to the lupan skeleton of betulinic acid.

Previous research had reported that the potentially active lupan was dependent on the presence of two hydroxyl functional group substituents in position C-3 and C-30[31]. These features seemed to be favourable for the antimicrobial activity. In this study, although betulinic acid has hydroxyl group in position C-3, the presence of carboxyl acid functional group substituent in position C-28 and the absence of hydroxyl group in position C-30 might be the cause for the loss of the antibacterial activity.

Table 1
The results of antibacterial property of tested extracts and compounds

Test Components µg/disc	Test bacteria and zone of inhibitions (mm)								
	SP	BC	SA	SPn	HI	ST	SF	KP	EC
MTE									
160	9±0.5	8	11±0.5	-	-	-	-	7±0.5	-
80	-	7	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-
ACE									
160	13	10±0.5	14	9	9±0.5	-	-	9	-
80	10±0.5	8	13	8±0.5	8±0.5	-	-	-	-
40	8	-	12	7	7	-	-	-	-
24	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-
Compound **1**									
20	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-	-
Compound **2**									
20	13±0.5	8	-	-	-	-	-	7	-
8	10	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-	-
Compound **3**									
20	12	7±0.5	-	-	-	-	-	7	-
8	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
0.5	-	-	-	-	-	-	-	-	-
Compound **4**									
20	12	7	NT	NT	-	-	-	NT	NT
8	10	-	NT	NT	-	-	-	NT	NT
4	-	-	NT	NT	-	-	-	NT	NT
2	-	-	NT	NT	-	-	-	NT	NT

1	-	-	NT	NT	-	-	-	NT	NT
0.5	-	-	NT	NT	-	-	-	NT	NT
TE (30)	39	NT	NT	NT	NT	NT	40	NT	NT
GN (10)	NT	NT	NT	NT	NT	NT	NT	17±0.7	23
CXM (30)	NT	NT	36±0.7	19±0.7	18	25±0.5	NT	NT	NT
AML (25)	NT	8±0.5	NT	NT	NT	NT	NT	NT	NT

SP: *S. pyogenes*, BC: *B. cereus*, SA: *S. aureus*, SPn: *S. pneumoniae*, HI: *H. influenzae*, ST: *S. typhimurium*, SF: *S. flexneri*, KP: *K. pneumoniae*, EC: *E. coli*; MTE: Methanol extract, ACE: Acetone fraction; TE: Tetracycline, GN: Gentamicin, CXM: Cefuroxime, AM: Amoxicillin; NT: Not tested, (-): No inhibition

Conclusion

In conclusion, all tested samples (extract and pure compounds) from *S. grandiflora* root assayed their activity against at least one strain of the tested aerobic bacteria. The acetone fraction exhibited stronger antibacterial activity than the methanol extract. The isolated isoflavanoid compounds showed promising antibacterial activity.

The results of this study justified the use of *S. grandiflora* root in traditional medicine to treat diseases caused by bacterial infection. This study also indicates that *S. grandiflora* root could be a potential source of new antibacterial agents.

Acknowledgement

The authors thank Universiti Sains Malaysia for providing fund (RU Grant: 1001/PKIMIA/811133). We would like to thank Kemenristek-DIKTI, Republic of Indonesia for the support. We also acknowledge Dr. David Larsen (University of Otago, New Zealand) for HRESIMS measurement, Ms. Anis Safirah Mohd Zahariluddin and Ms. Nabihah Mohd Zin for their assistance in the antimicrobial assay.

References

1. Cronk Q., Ojeda I. and Pennington R.T., Legume comparative genomics: progress in phylogenetics and phylogenomics, *Curr. Opin. Plant Biol.*, **9**, 99-103 **(2006)**

2. Vitor R.F., Mota-Filipe H., Teixeira G., Borges C., Rodrigues A.I., Teixeire A. and Paulo A., Flavonoids of an extract of *Pterospartum tridentatum* showing endothelial protection against oxidative injury, *J. Ethnopharmacol.*, **93**, 363-370 **(2004)**

3. Gebre-Mariam T., Neubert R., Schmidt P.C., Wutzler P. and Schmidtke M., Antiviral activities of some Ethiopian medicinal plants used for the treatment of dermatological disorders, *J. Ethnopharmacol.*, **104**, 182–187 **(2006)**

4. Watjen W., Kulawik A., Suckow-Schnitker A.K., Chovolou Y., Rohrig R., Ruhl S., Kampkotter A., Addae-Kyereme J., Wright C.W. and Passreiter C.M., Pterocarpans phaseollin and neurautenol isolated from *Erythrina addisoniae* induce apoptotic cell death accompanied by inhibition of ERK phosphorylation, *Toxicology*, **242**, 71-79 **(2007)**

5. Neto M.M., Neto M.A., Filho R.B., Lima M.A.S. and Silveira E.R., Flavanoids and alkaloids from leaves of *Bauhinia ungulate* L, *Biochem. System Ecol.*, **36**, 227-229 **(2008)**

6. Roosita K., Kusharto C.M., Sekiyama M., Fachrurozi Y. and Ohtsuka R., Medicinal plants used by the villagers of a Sundanese community, *J. Ethnopharmacol.*, **115**, 72-81 **(2008)**

7. Nakamura E.S., Kurosaki F., Arisawa M., Mukainaka T., Okuda M., Tokuda H., Nishino H. and Pastore Jr F., Cancer chemopreventive effects of constituents of *Caesalpinia ferrea* and related compounds, *Cancer Lett.*, **177**, 119-124 **(2002)**

8. Federici E., Touche A., Choquart S., Avanti O., Fay L., Offord E. and Courtois D., High isoflavone content and estrogenic activity of 25 year-old *Glycine max* tissue cultures, *Phytochemistry*, **64**, 717-724 **(2003)**

9. Ferreira D., Marais J.P.J. and Slade D., Review, Phytochemistry of the mopane, *Colophospermum mopane*, *Phytochemistry*, **64**, 31-51 **(2003)**

10. Palazzino G., Rasoanaivo P., Federici E., Nicoletti M. and Galeffi C., Prenylated isoflavonoids from *Millettia pervilleana*, *Phytochemistry*, **63**, 471-474 **(2003)**

11. Srinivas K.V.N.S., Koteswara Y.R., Mahender I., Das B., Krishna K.V.S.R., Kishore K.H. and Murty U.S.N., Flavanoids from *Caesalpinia pulcherrima, Phytochemistry*, **63**, 789-793 **(2003)**

12. Nozaki N., Hayashi K., Kido M., Kakumoto K., Ikeda S., Matsuura N., Tani H., Takaoka D., Iinuma M. and Akao Y., Pauferrol A, a novel chalcone trimer with cyclobutane ring from *Caesalpinia ferrea* mart exhibiting DNA Topoisomerase II inhibition and apoptosis-inducing activity, *Tetrahedron Lett.*, **48**, 8290-8292 **(2007)**

13. Anonymous, *Sesbania grandiflora* (L.) POIRET, Biodiversity in Medicinal and Aromatic Plants in India, Available from: http://www.impgc.com/test.php?id=Sesbania%20grandiflora **(2010a)**

14. Anonymous, *Sesbania grandiflora* (Katuray), Available from http://www.stuartxchange.com/Katurai.html. **(2010b)**

15. Doddola S., Pasupulati H., Koganti B. and Prasad K.V.S.R.G., Evaluation of *Sesbania grandiflora* for antiurolithiatic and antioxidant properties, *J. Nat. Med.*, **62**, 300-307 **(2008)**

16. Fojas F.R., Barrientos C.M., Capal T.V., Cruzada S.F., Sison F.M., Co Y.C., Chua N.G. and Gavina T.L., Preliminary phytochemical and pharmacological studies of *Sesbania grandiflora* (L.) Pers, *Phil. J. Sci.*, **111**, 157-181 **(1982)**

17. Kasture V.S., Deshmukh V.K. and Chopde C.T., Anxiolytic and anticonvulsive activity of *Sesbania grandiflora* leaves in experimental animals, *Phytother. Res.*, **16**, 455-460 **(2002)**

18. Pari L. and Uma A., Protective effect of *Sesbania grandiflora* against erythromycin Estolate-Induces hepatotoxicity, *Therapie*, **58**, 439-443 **(2003)**

19. Ramesh T. and Begum V.H., Effect of *Sesbania grandiflora* on lung antioxidant defense system in cigarette smoke exposed rats, *Int. J. Biol. Chem.*, **1**, 141-148 **(2007)**

20. Ramesh T. and Begum V.H., Protective effect of *Sesbania grandiflora* against cigarettes smoke-induced oxidative damage in rats, *J. Med. Food*, **11**, 369-375 **(2008)**

21. Goun E., Cunningham G., Chu D., Nguyen C. and Miles D., Antibacterial and antifungal activity of Indonesian ethnomedical plants, *Fitoterapia*, **76**, 592-596 **(2003)**

22. Hasan N., Osman H., Mohamad S., Chong W., Awang K. and Zahariluddin A.S.M., The chemical components of *Sesbania grandiflora* roots and their antituberculosis activity, *Pharmaceuticals*, **5**, 882–889 **(2012)**

23. Andrews J.M., BSAC standardized disc susceptibility testing method, *J. Antimicrob. Chemother.*, **48(Suppl. S1)**, 43-57 **(2001)**

24. Gao Y., Belkum M.J.V. and Stiles M.E., The Outer membrane of Gram-negative bacteria inhibits antibacterial activity of brochocin-C, *Appl. Environ. Microbiol.*, **65**, 4329-4333 **(1999)**

25. Nostro A., Germano M.P., D'Angelo V., Marino A. and Cannatelli M.A., Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity, *Lett. Appl. Microbiol.*, **30**, 379-384 **(2000)**

26. Black J.G., Characteristics of prokaryotic and eukaryotic cells, In Microbiology: Principles and Explorations, 7th Edition, John Wiley and Sons, Asia, 83-90 **(2008)**

27. Tadeg H., Mohammed E., Asres K. and Gebre-Mariam T., Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders, *J. Ethnopharmacol.*, **100**, 168-175 **(2005)**

28. Bojase G., Majinda R., Gashe B.A. and Wanjala C.C.W., Antimicrobial flavonoid from *Bolusanthus speciosus*, *Planta Medica*, **68**, 615-620 **(2002)**

29. Sato M., Tanaka H., Fujiwara S., Hirata M., Yamaguchi R., Etoh H. and Tokuda C., Antibacterial property of isoflavonoids isolated from *Erythrina variegate* against cariogenic oral bacteria, *Phytomedicine*, **9**, 427-433 **(2002)**

30. Koysomboon S., Altena I.V., Kato S. and Chantrapromma K., Antimycobacterial flavonoids from *Derris indica*, *Phytochemistry*, **67**, 1034-1040 **(2006)**

31. Mutai C., Bii C., Vagias C., Abatis D. and Roussis V., Antimicrobial activity of *Acacia mellifera* extracts and lupine triterpenes, *J. Ethnopharmacol.*, **123**, 143-148 **(2009)**.

(Received 22nd October 2019, accepted 16th December 2019)