Chemical Composition and *in vitro* Antimicrobial Properties of *Phyllanthus columnaris* Stem Bark Tannins Against Oral Pathogens

T. A. M. Othman[1], Rohazila Mohd Hanafiah[2], Noor Akmar Nam[2], Shahida Mohd-Said[3], Siti Noor Adnalizawati Adnan[2]*

1. Faculty of Science and Technology, Universiti Sains Islam Malaysia, Bandar, Negeri Sembilan, Malaysia.
2. Faculty of Dentistry, Universiti Sains Islam Malaysia, Kuala Lumpur, Malaysia.
3. Centre for Restorative Dentistry, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

Abstract

The potential antimicrobial properties of tannins from *Phyllanthus columnaris* stem bark were evaluated against three Gram-positive cariogenic bacteria (*Streptococcus salivarius* ATCC 13419, *Streptococcus oralis* ATCC 6249 and *Streptococcus mutans* ATCC 25175), two obligate anaerobic Gram-negative periodontopathic bacteria (*Porphyromonas gingivalis* ATCC 33277, and *Fusobacterium spp* ATCC 25586), and five *Candida* spp (*C. albicans* ATCC 14053, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 and *C. glabrata* ATCC 2001). Antimicrobial activities of tannins were determined using the disc diffusion test as well as minimum inhibitory concentration and minimum bactericidal/fungicidal concentration tests. The chemical composition of tannins was analysed by direct infuse mass spectrometry. Tannins inhibited the growth of all tested pathogens at MIC value ranging from 0.16 to 1.25 mg/mL. All tested bacteria showed similar higher level of susceptibility against tannins as the lowest MIC value was detected (0.16 mg/mL) except for *S. oralis* (0.63 mg/mL). However, both Gram negative anaerobes recorded the lowest MBC value at 0.32 mg/mL as compared to the Gram positive cariogenic bacteria (2.5->5.0 mg/mL). DIMS analysis of precursor ions detected four compounds present in tannins namely punicafolin, (S)-Nerolidol 3-O-[a-L-Rhamnopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-[4-(4-hydroxy-3 methoxycinnamoyl)-(E)-a-L-rhamnopyranosyl-(1->6)]-b-D-glucopyranoside], ent-Epicatechin-(4alpha->8)-ent-epicatechin-(4alpha->8)-ent-epicatechin 3',3''-digallate and pavetannin C1 respectively.

Experimental article (J Int Dent Med Res 2019; 12(3): 848-853)

Keywords: Oral pathogens, Tannins, Antimicrobial, DIMS.

Received date: 15 July 2018 **Accept date:** 27 October 2018

Introduction

Oral diseases continue to be one of the major health problems worldwide.[1] Dental caries and periodontal diseases are among the most important global oral health problems in addition to chronic conditions such as oral and pharyngeal cancers and also oral tissue lesions which are already known to imply significant health concerns.[2]

Oral Streptococci, the main causative agents of the oral infectious diseases,[3] are commonly presented on the tooth surface as biofilm or dental plaque. This oral biofilm plays an important role in the initiation, development and progression of dental caries and periodontal diseases.[4]

Despite some advances in various fields of medicine, dental caries and other oral infectious diseases are still considered as serious health problems and inflict major burden to health care services and worldwide populations especially in the developing countries.[5-6] Besides, complications like the emergence of resistance against antibiotics and chemotherapeutics is a growing cause of concern which have limited the patient management procedures and also preventive measures. Therefore, there is a continuing need to search for new alternative antimicrobial agents.[7]

The global need for alternative prevention and treatment options for oral diseases that are safer, effective and economical are due to the

Corresponding author:
Siti Noor Adnalizawati Adnan
Faculty of Dentistry,
Universiti Sains Islam Malaysia,
Kuala Lumpur, Malaysia.
E-mail: adnaliza@usim.edu.my

rise in disease incidence (particularly in developing countries), resistance phenomena by pathogenic bacteria to currently used antibiotics and chemotherapeutics, opportunistic infections in immunocompromized individuals and financial considerations in developing countries.[8-9] Despite several therapeutics agents being commercially available, these chemicals were reported to cause undesirable side-effects such as vomiting, diarrhea, tooth staining and altering the oral microbiota.[10-11]

Since decades and until recently, medicinal plants have been used traditionally in treating variety of human diseases globally especially in the rural areas of the developing countries.[12] About 80% of the people in developing countries utilize herbal medicines for health care purposes. Tannins, which is one of the natural plant products have been revealed to possess significant broad spectrum of antimicrobial activity against several pathogens including antibacterial and antibiofilm activities.[13] Tannins from *Phyllanthus columnaris* stem bark exhibited remarkable inhibitory effects on a broad range of pathogenic microorganisms including methicillin resistance *Staphylococcus aureus* (MRSA).[14] In view of this, we aimed to analyse the chemical composition of tannins and also further explore and scrutinize its efficacy on oral pathogens in order to develop tannins as one of the new alternative source of highly effective intervention for oral infectious diseases.

Materials and methods

Preparation of tannins

Tannins from *Phyllanthus columnaris* stem bark were obtained from previous study.[15] Tannins were diluted in dimethyl sulfoxide (DMSO) to a final concentration stocks of 100 mg/mL for disc diffusion assay and 20 mg/mL for the assay in determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)/ minimum fungicidal concentration (MFC) test. All diluted tannins were sterilized by filtration through a 0.45 µm membrane filter.

Microorganisms and materials

The microorganisms used in this study were three facultative anaerobic Gram-positive cariogenic bacteria namely *Streptococcus salivarius* ATCC 13419 *Streptococcus oralis* ATCC 6249, *Streptococcus mutans* ATCC 25175; two obligate anaerobic Gram-negative periodontopathic bacteria (*Porphyromonas gingivalis* ATCC 33277 and *Fusobacterium spp* ATCC 25586) and also five *Candida* spp. (*C. albicans* ATCC 14053, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 and *C. glabrata* ATCC 2001). All bacteria strains were grown and maintained on brain heart infusion agar (BHIA) slopes (Oxoid Ltd., Basingstoke, UK) for Gram-positive bacteria and supplemented-brain heart infusion agar (S-BHI) slopes for Gram-negative bacteria. All yeasts were grown and maintained on potato dextrose agar (PDA) slopes (Oxoid Ltd., Basingstoke, UK). Mueller–Hinton agar (MHA) (Oxoid Ltd., Basingstoke, UK), Mueller–Hinton broth (MHB) (Oxoid Ltd., Basingstoke, UK), PDA (Oxoid Ltd., Basingstoke, UK) and potato dextrose broth (PDB) (Oxoid Ltd., Basingstoke, UK) were used as growth media for antimicrobial assays.

Screening of antimicrobial activity

Disc diffusion method for antimicrobial screening test was carried out according to Clinical and Laboratory Standards Institute (2006)[16] to assess the potential antimicrobial activities of tannins from *Phyllanthus columnaris* stem bark. Approximately, 10^8 CFU/mL inoculum of each tested bacteria was grown on BHI plate evenly using a sterile swab. Inoculum of candidas were prepared at 10^6 CFU\ml prior to evenly swabbed on PDA. All inoculated plates were dried at room temperature for 5 min. The discs which had been impregnated with a series of tannins dissolved in different solvents [sterile distilled water, phosphate buffer saline (PBS) and DMSO] were placed on the inoculated BHIA surface. The standard antibiotic discs which were tetracycline 30 µg and ampicillin 10 µg were used as positive controls for bacteria whereas fluconazole 25 µg acted as positive control for *Candida* spp. Sterile distilled water, PBS and DMSO were used as negative controls. All the plates were then incubated at 37°C for 24 h. After the incubation, the presence of inhibition zone were examined and its diameter were measured in mm. This assay was repeated three times to ensure reliability.

Determination of MIC, MBC and MFC values

The MICs of tannins against tested oral pathogens were determined using broth

microdilution method on 96-well sterile microtiter plates as described by Basri et al. (2012)[17] with some modifications. Approximately 50 µL of the prepared tannin was added to the first well containing 50 µL of sterile BHI broth (for Gram-positive bacteria) or S-BHI broth (for Gram-negative bacteria) or PDB for *Candida* spp and serially diluted by two-fold. From the tenth well, 50 µL of solution was pipetted out and disposed off. Subsequently, 50 µL of microbial suspension containing 10^6 CFU/mL was added into the first until the eleventh well, resulting in final concentrations of tannins in DMSO ranging from 5 mg /mL to 0.009 mg/mL (from the first well to the tenth well). The eleventh wells were used as inoculum control, containing 50 µL of BHI (for Gram-positive bacteria) or S-BHI broth (for Gram-negative bacteria) or PDB for *Candida* spp and 50 µL of microbial suspension. The twelfth wells acted as positive control which contained 50 µL of broth (BHI or S-BHI, PDB) and microbial suspension and also 50µL of ampicillin (10 mg/mL). The H wells (from the first well to the tenth well) contained a series of tannins ranging from 5 mg /mL to 0.009 mg/mL in (BHI or S-BHI) or PDB to serve as negative control for turbidity reference.

The microtiter plates were then incubated at 37°C with 5% CO_2 for 24 h (for Gram-positive bacteria) or 48 h (for Gram-negative bacteria) in an anaerobic jar. A dye consisted of 20 µL 3-(4,5-dimethyl-2-thiazolyl) 2,5-diphenyl-2H-tetrazolium bromide (MTT, 1 mg/mL) (Merck, Germany) 40 µL 2,3,5-triphenyltetrazolium chloride (TTC, 2 mg/mL) (Merck, Germany) was added as an indicator to aid in determining the growth of Gram positive and Gram-negative bacteria respectively. The plate was incubated for another 2 hours in the dark. The lowest concentration of tannins with colour changes detected in the well were considered as the MIC value (no visible growth of the tested bacteria. The microtiter plates of *Candida* strains were incubated at 30°C for 24 h. The MIC value was determined based on the turbidity in which is related to the rate of fungal growth.

Following the determination of the MIC value, each well that did nto show any visible changes of the indicator (starting from the well with MIC value and upward) was transfered onto BHI or S-BHI agar. All plates then were incubated at 37°C for 24 h (for Gram positive bacteria) and 48 h (for Gram negative bacteria). The least concentration which showed no visible growth on the agar plate after incubation period was considered as the MBC value. Meanwhile, MFC value was determined by subculturing sample from each well which showed no visible fungal growth onto PDA plate and incubated at 30°C for 24 h. The least concentrations which showed no growth of fungal on agar plate was recorded as MFC values.

Chemical composition analysis

Analysis of the chemical composition of tannin was carried out using the direct infuse mass spectrometry (DIMS). The tannins sample was directly injected through a syringe pump (Havard apparatus, 11 plus, USA) at flow rate 10 uL/min into the electrospray ionization source (ESI) without going through chromatographic separation. The mass spectrometry detection was performed using a ACQUITY® SQD with Single Quadrupole Detector (Waters Corporation, Milford, MA USA) operated in positive and negative ion electrospray modes. Nitrogen was used as desolvation gas. Data acquisition was performed from 50 to 1500 Da with source temperature set at 120°C, desolvation temperature set at 250°C, extractor at 3 V, cone gas flow set at 50 L/h, capillary voltage in positive and negative mode at 3.0 kV and 2.8 kV respectively, desolvation gas flow was at 500 L/h for positive mode and 550 L/h in negative mode. The cone voltage was set at 50 V for positive mode and 30 V for negative mode. A centroid data collection mode was used in this analysis and the mass spectrometry system were controlled by MassLynx 4.1 software (Waters).

Results

Antimicrobial activity of tannins on oral pathogens

Tannins dissolved in DMSO exhibited strongest inhibitory effect compared to tannins dissolved in other two diluents which were sterile distilled water and PBS (figure 1). The results of the antimicrobial activity of tannins from *Phyllanthus columnaris* stem bark against tested oral pathogens were tabulated in Table 1.

Oral Pathogens	Inhibition zone diameter (Mean ± SD)				MIC (mg/mL)	MBC/MFC (mg/mL)
	Tetracycline 30 µg	Ampicillin 10 µg	Fluconazole 25 µg	Tannins in DMSO 100 mg/mL		
Antibacterial activities						
S. mutans	35 ± 0.1	20 ± 0.1	×	13 ± 0.2	0.16	2.5
S. salivarius	26 ± 0.2	40 ± 0.0	×	14 ± 0.7	0.16	2.5
S. oralis	17 ± 0.2	17 ± 0.2	×	12 ± 0.5	0.63	>5
P. gingivalis	×	×	×	×	0.16	0.31
F. nucleatum	×	×	×	×	0.16	0.31
Antifungal activities						
C. albicans	×	×	23±0.0	10 ± 0.5	1.25	5
C. parapsilosis	×	×	14±0.1	11 ± 0.5	1.25	5
C. tropicalis	×	×	5±0.2	17 ± 0.7	0.63	2.5
C. krusei	×	×	5±0.0	9± 0.7	0.63	2.5
C. glabrata	×	×	14±0.3	20 ± 0.7	0.63	2.5

×: Not tested

Table 1. The Antimicrobial Activities of Tannins from the Disc Diffusion Assay (the Mean Diameter of Inhibition Zones, Mm), and Also the MIC and MBC/MFC Values.

Screening of the antifungal activity from the disc diffusion assay showed that tannins diluted in DMSO demonstrated the strongest inhibitory effect towards *C. glabrata* with diameter of inhibition zone detected was 20 ± 0.7 mm. In contrast, *C. krusei* was the least susceptible indicated by the smallest diameter of inhibition zone recorded (9 ± 0.7 mm). Among all the oral bacteria tested, *S. salivarius* was found to be the most susceptible towards tannins as it displayed the largest inhibition zone (14 ± 0.7 mm) as compared to the other bacteria, whereas the smallest inhibition zone was detected against *S. oralis* (12 ± 0.5 mm). Nevertheless, the diameter of inhibition zones showed against all tested bacteria were significantly smaller in comparison to the inhibition zone showed by the positive control used in this study, Tetracycline and Ampicillin.

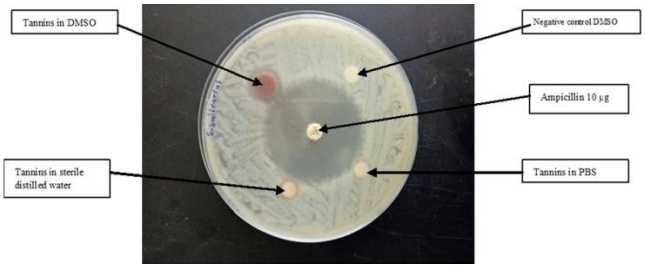
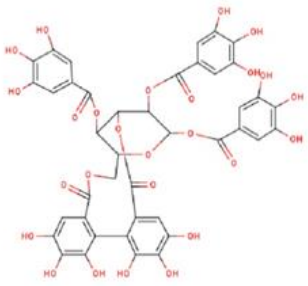


Figure 1. A Representative Picture of Antimicrobial Screening Test of Tannins Against Selected Oral Pathogen.

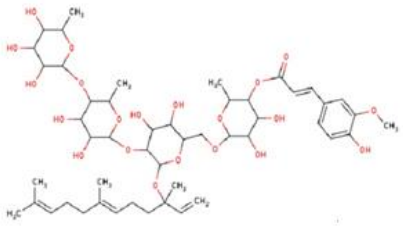
The MIC values of tannins from *Phyllanthus columnaris* stem bark detected against tested oral pathogens ranging from 0.16 to 1.25 mg/mL (Table 1). Tannins showed the lowest similar MIC value of 0.16 mg/mL against all tested bacteria except for *S. oralis* (0.63 mg/mL). For fungi, the MIC values were determined based on the turbidity of the mixture (related to the rate of fungal growth). In this study, tannins showed a moderate inhibitory effect towards two fungal species namely *C. albicans* and *C. parapsilosis* with MIC value of 1.25 mg/ml whereas *C. tropicalis, C. glabrata* and *C. krusei* exhibited higher susceptibility against tannins at MIC value of 0.16 mg/mL.

The MBC and MFC values of tannins from *Phyllanthus columnaris* stem bark against oral pathogens were also demonstrated in Table 1.

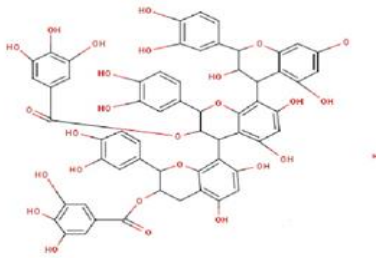
Tannins displayed the highest MBC value (> 5 mg/mL) against *S. oralis* and the lowest MBC values towards both anaerobes tested in this study (0.32 mg/mL). As for MFC, higher value was detected against *C. albicans* and *C. parapsilosis* at 5.0 mg/mL whilst other *Candida* spp recorded the same lower value of 2.5 mg/mL.



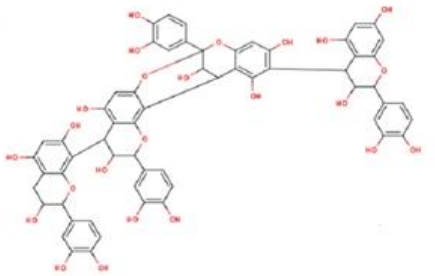
A



B



C



D

Figure 2. Chemical Structure of **A.** Punicafolin. **B.** (S)-Nerolidol 3-O-[a-L-Rhamnopyranosyl-(1->4)-a-L-Rhamnopyranosyl-(1->2)-[4-(4-Hydroxy-3-methoxycinnamoyl)-(E)-a-L-Rhamnopyranosyl-(1->6)]-b-D-Glucopyranoside]. **C.** Ent-Epicatechin-(4alpha->8)-Ent-Epicatechin-(4alpha->8)-Ent-Epicatechin 3',3''-digallate and **D.** Pavetannin C1.

Chemical composition of tannins

DIMS analysis of precursor ions detected four compounds by Single Quadrupole Detector in tannins with *m/z* of 939.10, 1,043.44, 1,171.23 and 1,185.28 respectively. The molecular formulas of the compounds were determined as $C_{41}H_{30}O_{26}$, $C_{49}H_{74}O_{21}$, $C_{59}H_{46}O_{26}$ and $C_{60}H_{48}O_{24}$ respectively. Compound 1 has been identified as Punicafolin, compound 2 as (S)-Nerolidol 3-O-[a-L-Rhamnopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-[4-(4-hydroxy-3 methoxycinnamoyl)-(E)-a-L-rhamnopyranosyl-(1->6)]-b-D-glucopyranoside], compound 3 as ent-Epicatechin-(4alpha->8)-ent-epicatechin-(4alpha->8)-ent-epicatechin 3',3''-digallate and compound 4 as Pavetannin C1 (figure 2 a-d)

Discussion

In this study, different diluents which were sterile distilled water, PBS and DMSO were used in the preliminary screening of antimicrobial activity of tannins in order to compare their capability as a diluent in aiding the diffusion of tannins. DMSO was proven as the most suitable diluents by exerting the largest inhibition zone detected in the disc diffusion assay. DMSO is a polar aprotic solvent which dissolves both polar and non-polar compounds. It is also miscible with water which enables its diffusion on the agar surface to be better than the other solvents.

In general, results from the antimicrobial screening showed significant differences of inhibition towards the *Candida* spp. ranging from 9 ± 0.7 to 20 ± 0.7 mm in diameter whilst no significant differences were shown towards all of the oral bacteria tested. Among all of the pathogens tested, tannins at 100 mg/mL demonstrated the largest inhibition zone towards *C. glabrata* (20 ± 0.7 mm in diameter). However, the recorded MIC and MBC/MFC values defined different interpretation as compared to the disc diffusion assay because the lowest MIC values (0.16 mg/mL) were detected against all the tested bacteria except for *S. oralis*. Tannins also displayed the lowest MBC value of 0.32 mg/mL towards both tested anaerobes.

In order to characterize the types of antibacterial activities of a plant compound, the determination of the MIC and MBC/MFC values are necessary to compare their significant activities. The evaluation of bacteriostatic or bactericidal activity exhibits by a plant compound is important in the development of new antimicrobial agent.[18] Our study demonstrated the differences in MIC or MBC/MFC value of tannins towards all tested pathogens including bacteria and fungi. This is possibly due to several contributing factors either related to the pathogens or tannins. All tested oral pathogens including Gram positive and Gram negative bacteria and also the fungi of *Candida* spp. have different anatomical structures of cell wall and cell membrane which act as the first barrier for

the pathogens in defending themselves from the mode of antmicrobial action of tannins. Furthermore, the physiological functions of each component constituting the membrane and cell wall of each pathogen may contribute to the differences in its susceptibility level against tannins.

The physicochemical properties of tannins also play important roles in its antimicrobial effects including the polarity of the active compounds. This study have revealed the presence of punicafolin, (S)-Nerolidol 3-O-[a-L-Rhamnopyran osyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-[4-(4-hy droxy-3-methoxycinnamoyl)-(E)-a-L-rhamnopyra nosyl-(1->6)]-b-D-glucopyranoside],ent-Epicatec hin-(4alpha->8)-ent-epicatechin-(4alpha->8)-ent-epicatechin 3',3''-digallate and pavetannin C1 in tannins. All of the compounds detected have been scarcely reported on their antimicrobial activity especially against oral pathogens except for ent-Epicatechin-(4alpha->8)-ent-epicatechin-(4alpha->8)-ent-epicatechin 3',3''-digallate. A study done by Morin et al (2015),[19] reported that this phytochemical compound which is one of the major components of the green tea extract inhibited the growth of *Solobacterium moorei* at the MIC and MBC values of 500 and 250 µg/ml, respectively. Besides, the antimicrobial activity of tannins also maybe contributed and enhanced by the presence of various phytochemical compounds that possess antimicrobial activity.

Conclussion

Tannins from the stem bark of *Phyllantus columnaris* possess bacteriostatic, bactericidal and fungicidal activities against oral Gram positive and Gram negative bacteria and also fungi (*Candida* spp.) the findings from this study suggest its potential to be developed as new antimicrobial agent in the management of oral infectious diseases such as dental caries and periodontal diseases. However, further investigations are needed to deeply explore the inhibitory effect of tannins against oral pathogens at cellular and molecular levels through *in vitro* and *in vivo* studies.

Acknowledgements

The authors would like to thank Universiti Sains Islam Malaysia for the financial support under the grant number PPP/USG-0215/FPG/30 /16715.

References

1. Petersen PE. The Burden of Oral Disease: Challenges To Improving Oral Health In The 21st Century. Bull World Health Organ. 2005;83(1):3.
2. Petersen PE. The World Oral Health Report 2003: Continuous Improvement of Oral Health in the 21st Century—The Approach of The WHO Global Oral Health Programme. Community Dent Oral Epidemiol. 2003;31:3–24.
3. Hirasawa M, Takada K. Susceptibility of Streptococcus mutans And Streptococcus sobrinus To Cell Wall Inhibitors and Development of a Novel Selective Medium for S. Sobrinus. Caries Res. 2002;36(3):155-160.
4. Koo H, Gomes BPFA, Rosalen PL, Ambrosano GMB, Park YK, Cury JA. In vitro Antimicrobial Activity of Propolis And Arnica montana Against Oral Pathogens. Arch Oral Biol. 2000;45:141-148.
5. Singh J, Kumar A, Budhiraja S, Hooda A. Ethnomedicine: Use in Dental Caries. Braz J Oral Sci. 2007;6:1308–1312.
6. Poole K. Overcoming Antimicrobial Resistance by Targeting Resistance Mechanisms. J Pharmacy Pharmacol. 2001;53:283–284.
7. Cai L, Wu CD. Compounds from Syzygium aromaticum Possessing Growth Inhibitory Activity Against Oral Pathogens. J Nat Prod. 1996;59:987–90.
8. Tichy J, Novak J. Extraction, Assay, and Analysis of Antimicrobials from Plants with Activity Against Dental Pathogens (Streptococcus sp.) J Altern Complement Med. 1998;4(1):39–45.
9. Badria FA, Zidan OA. Natural Products for Dental Caries Prevention. J Med Food. 2004;7(3):381–384.
10. Park KM, You JS, Lee HY, Baek NI, Hwang JK. Kuwanon G. An Antibacterial Agent from The Root Bark of Morus alba Against Oral Pathogens. J Ethnopharmacol. 2003;84(2-3):181–185.
11. Chung JY, Choo JH, Lee MH, Hwang JK. Anticariogenic Activity of Macelignan Isolated from Myristica fragrans (Nutmeg) Against Streptococcus mutans Phytomedicine. 2006;13(4):261–266.
12. Chitme HR, Chandra R, Kaushik S. Studies on Anti-Diarrhoeal Activity of Calotropis gigantea R. Br. In Experimental Animals. J Pharm Pharm Sci. 2003;7:70–75.
13. Kim H-S. Do Not Put Too Much Value on Conventional Medicines. J Ethnopharmacol. 2005;100(1-2):37–39.
14. Siti-Noor-Adnalizawati A, Angeline S, Nazlina I, Yaacob WA. Antimicrobial Activity of Phyllanthus columnaris Stem Bark Extract. Malays Appl Biol. 2013;42(1):83-87.
15. Siti NAA, Nazlina I, Yaacob WA. Isolation and Identification of Anti-Methicillin Resistant Staphylococcus Aureus Compounds from Phyllanthus columnaris Stem Bark. Malays J Microbiol. 2014;10:225-233.
16. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Test. Approved Standard. 9th ed. Wayne PA USA: CLSI;2006:15-36.
17. Basri DF, Tan LS, Shafiei Z, Zin NM. In vitro Antibacterial Activity of Galls of Quercus infectoria Olivier Against Oral Pathogens. Evidence-based Complementary Altern Med. 2012;1-6.
18. Grare M, Fontanay S, Cornil C, Finance C, Duval RE. Tetrazolium Salts for MIC Determination in Microplates: Why? Which Salt to Select? How? J Microbiol Methods. 2008;75:156–159.
19. Morin MP, Bedran TB, Fournier-Larente J, Haas B, Azelmat J, Grenier D. Green Tea Extract and Its Major Constituent Epigallocatechin-3-Gallate Inhibit Growth and Halitosis-Related Properties of Solobacterium moorei. BMC Complement Altern Med. 2015;15(48):1-11.