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Antibacterial activity of *Acmella paniculata* extracts against *Streptococcus mutans*

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ABSTRACT

Acmella paniculata, also known as 'Subang nenek' in Malaysia, has been used to treat diseases such as toothache and gum infections. People called it a toothache plant, and it has been widely used as traditional medicine. Therefore, this study aims to investigate the antibacterial activities of *A. paniculata* leaves and flowers extracts towards *Streptococcus mutans* by using disc diffusion assay, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) methods. Besides, the anti-biofilm activity of all the extracts also been determined by using a crystal violet assay. As the results, n-hexane and methanol extracts from leaves showed the highest inhibition zone towards *S. mutans* when compared to DCM and acetone extracts. Meanwhile, for the flowers extract, n-hexane and DCM showed the highest inhibition zone towards *S. mutans* compared to methanol and acetone extracts. The best results were then tested for MIC and MBC tests. As for the MIC values of n-hexane and methanol leaves extracts were 25 mg/mL, respectively, and the MBC values were 50 and 100 mg/mL, respectively. Whereas MIC values for n-hexane and DCM flowers extracts were 12.5 mg/mL, respectively, and the MBC values were 50 mg/mL, respectively. Biofilm formation of *S. mutans* showed decrement up to 70% after exposure to both leaves extract (n-hexane and methanol) and n-hexane flower extract. Still, it differed when exposing to DCM flower extract, and the result showed that the biofilm activities of *S. mutans* were inhibited at 80% after treated with DCM flowers extracts. In conclusion, n-hexane leaves extract, methanol leaves extract, n-hexane flowers extract, and DCM flowers extract of *A. paniculata* demonstrated bactericidal properties against *S. mutans.*

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INTRODUCTION

Dental caries is a major dental health problem affecting humankind that arises from colonization and accumulation of oral bacteria, especially *Streptococcus mutans*. Dental caries is a process of demineralization of tooth structure in the present of the tooth surface, pellicle, time and germs. *S. mutans* is gram-positive cocci shaped bacteria and grows at a temperature of 18-40^{o}c (Ong *et al.*, 2011). These bacteria are a cariogenic microorganism that breaks down sugar for energy and produces acidic conditions, which causes the demineralization of the superficial structure of the tooth $. The *S. mutans* is the main culprit of this disease.

Table 1: The inhibition zone of *S. mutans* when treated with different concentration of *A. Paniculata* leaves extract, DMSO and NaF

Sample Extracts (Leaves)	Inhibition zone Concentration (mg/mL) 12.5	25	50	100	DMSO	Naf 1 mg/mL
-hexane***	6.0±0.0a	8.0±0.001b	9.0±0.001c	10.0±0.001d	6.0±0.0a	15.0±0.0
DCM	6.0±0.0a	6.0±0.0a	6.0±0.0a	6.0±0.0a	6.0±0.0a	15.0±0.0
Acetone*	6.3±0.6a	6.3±0.6a	6.3±0.6a	6.3±0.6a	6.0±0.0a	15.0±0.0
Methanol**	6.0±0.0a	6.0±0.0a	7.0±0.001b	8.0±0.001c	6.0±0.0a	15.0±0.0

Table 2: The inhibition zone of *S. mutans* when treated with different concentration of *A. Paniculata* flowers extract, DMSO and NaF

Sample Extracts (Flowers)	Inhibition zone Concentration (mg/mL) 12.5	25	50	100	DMSO	Naf 1 mg/mL
-hexane*	7.5± 0.50a	8.67± 1.15 a,b	7.5± 0.50 b,c	8.67± 1.15c	6.0±0.0a	15.0±0.0
DCM **	9.67± 0.57a	10.33± 1.53b	10.67± 2.08c	12.33± 2.30d	6.0±0.0a	15.0±0.0
Acetone***	6.0± 0.001a	6.0± 0.001a	6.0± 0.001a	6.0± 0.001a	6.0± 0.001a	15.0±0.0
Methanol**	6.0±0.0a	6.0±0.0a	6.0±0.001a	8.0±0.001a	6.0±0.0a	15.0±0.0

Hence more attention is given to *S. mutans* in finding any material that has the antimicrobial effect toward this bacterium to prevent the formation of dental caries. The method that had been used in caries prevention is applied dental varnish on tooth surfaces. It contains fluoride as its active agent. Fluoride is anti-cariogenic properties by remineralization the enamel and minimizes the demineralizing action of bacteria which inhibit acid production of *S. mutans*. However, fluoride is not a potent antimicrobial agent. The previous study reported that fluoride varnish not efficiently to reduce in-vitro biofilm of *S. mutans* (Liao *et al.*, 2017).

This situation is increasingly challenging when other commonly used antibiotics and chemotherapeutics such as penicillin, cephalosporin, erythromycin and tetracycline have begun to be less useful to oral bacteria (Chandad and Grenier, 2007). Besides, (Teles and Teles, 2009) stated that prolonged used of antimicrobials agent as an additive to control plaque mechanism caused two major safety issues which were, development of resistant microorganisms and the risk of oral cancer related to the alcohol content in the mouth rinse formulations. Therefore, the researcher needs to find new alternative drugs which better and more efficient as compared to the current medication.

Traditional medicine treatment, such as herb is a common practice in many countries at the healthcare level. Many people believe that herbal remedies are safer as it is a natural product from the plant. Plants rich in secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Das *et al.*, 2010). Therefore, the demand of bioactive compound from the plant is continuously increasing to resolve multidrug resistance issue. The genus Acmella Rich (Asteraceae) comprises 39 species that could be found in the tropical and subtropical regions.

Acmella paniculata is an annual herb with 32-60 cm high. It has a lot of glandular and hairy stems with marigold eye flowers (Varghese *et al.*, 2014). In Malaysia, Acmella plant is used as a vegetable, and it is generally known as 'Subang nenek' where the flowers and the leaves have a pungent taste when consumed, caused tingling and numbness (Nomura *et al.*, 2013; Ong *et al.*, 2011).

People believe that this plant is useful for toothache, sore throat and gum infections. Traditionally, the flowers are chewed to get its effect, whereas the pounded plant is used for rheumatism (Estari and Gujjeti, 2013). Due to its growing use, it is vital to determine the antimicrobial activities of *A. Paniculata* extract against oral bacteria. Therefore, this study aims to identify the antibacterial activity of *A.*

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Table 3: MIC and MBC Values of n-Hexane and Methanol Leaves Extract and n-Hexane and DCM Flower Extract

Samples	MIC (mg/mL)	MBC (mg/mL)
n-hexane leaves extract	25	50
Methanol leaves extract	25	100
n-hexane flower extract	12.5	50
DCM flower extract	12.5	50

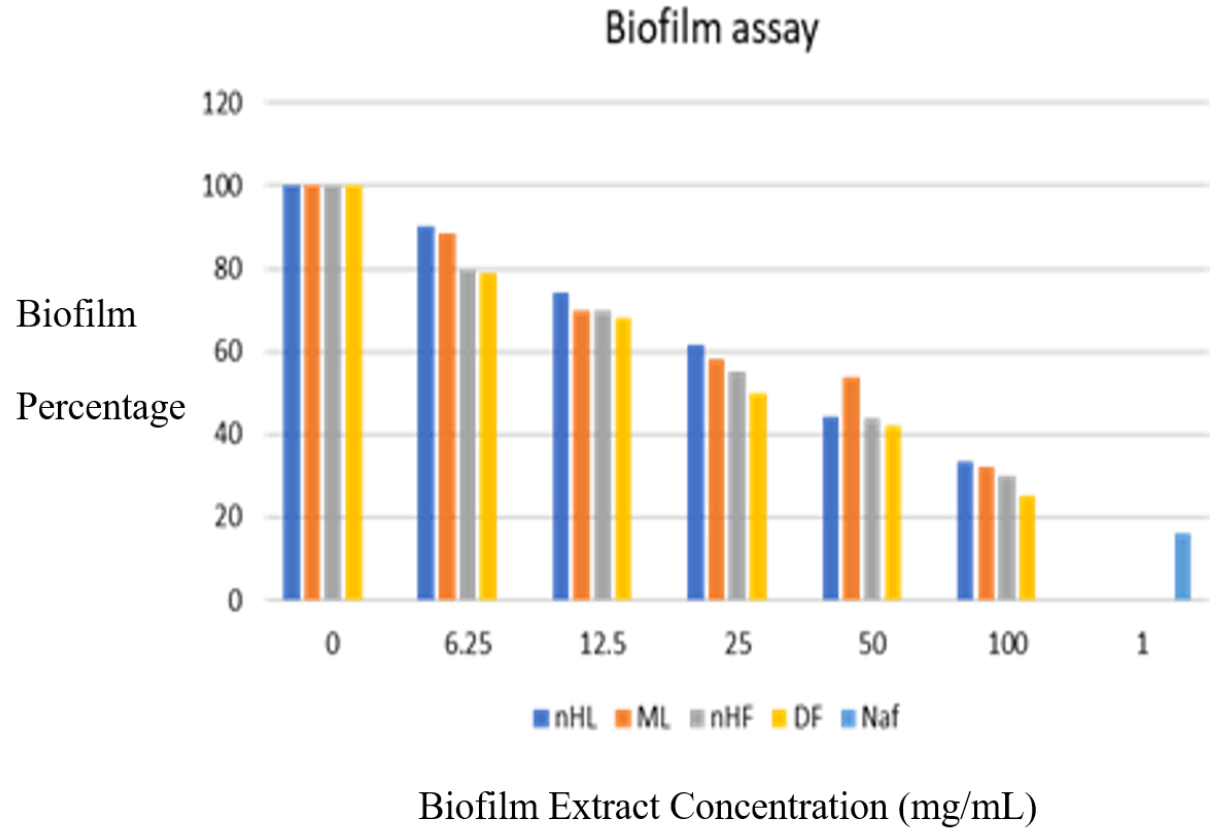

Figure 1: Methanolgure 1: Biofilm. N-hexanes-hexane leaves extract, and methanol leaves extract, n-hexane flowers extract, DCM flowers extract and Naf (1mg/mL) against *S. mutans*

paniculata extract against

S. mutans.

MATERIALS AND METHODS

Sample of *A. Paniculata*

A. paniculata was collected from TKC Herbal Nursery, Seremban (Malaysia). Botanist confirmed the plant, Dr Mohd Firdaus Ismail from Institute of Bioscience, University Putra Malaysia and the voucher specimen was deposited at the Herbarium of Institute of Bioscience, University Putra Malaysia. The voucher specimen number was MFI 0164/20.

Sample preparation

Flower and leaves of *A. paniculata* were dried at room temperature and pounded to a fine powder (Model C14, Kesmac Sdn. Bhd., Malaysia) then the samples were stored in black containers. Serial extraction was done to the samples (800 g) with 1 L of *n*-hexane, dichloromethane (DCM), acetone and methanol (System, Shah Alam, Malaysia) (Shai *et al.*, 2008).

After that, the extracts were filtered by using Whatman No. 1 filter paper and evaporated by using a rotary evaporator (Laborota 4000, Germany). Each extract was diluted in 10% DMSO (System, Shah Alam, Malaysia). *S. mutans* strain (ATCC 25175) was used in this study. *S. mutans* were grown on the brain–heart infusion agar (BHIA) and brain–heart infusion broth (BHIB) (Oxoid Ltd., Basingstoke, UK).

Disc Diffusion assay

S. mutans was cultured on BHIA at 37°C for 24 hours under anaerobic condition. After that, the bacterial culture was optimized to 0.5 McFarland with Muller Hinton broth (MHB). *S. mutans* were spread on Muller Hinton agar (MHA) using a sterile cotton swab. Disc infused with the flower and leaves extracts (10-30 mg/mL) were placed on top of MHA. Sodium fluoride (1 mg/mL NaF) (Sigma-Aldrich, Missouri, USA) and 10% DMSO were acting as a positive and negative control, respectively. Zones of inhibition were measured after incubation at 37°C for 24 hours under anaerobic condition. All tests were done in triplicate.

Minimum Inhibition Concentration (MIC) and Minimum bactericidal concentration (MBC)

MIC and MBC assay had been performed by followed method from (Ong *et al.*, 2011). MIC was done by using 96 well plates with two-fold serial dilution method. *S. mutans* (100 μL) at 10^8 CFU/mL was added to various concentrations of extracts (40.00 to 0.04 mg/mL) diluted in MHB to a final volume of 200 μL/well. DMSO 10% (v/v) was used as a negative control, and NaF (Sigma-Aldrich, Missouri, USA) was used as a positive control (CLSI, 1999). Following incubation at 37°C under the anaerobic condition for 24 h, the MIC value was determined as the lowest concentration that inhibits the visible growth of bacteria.

Minimum bactericidal concentration (MBC) was established by culturing a five μL aliquot from wells that exhibited no bacterial growth in MIC onto sterile MHA and incubated overnight at 37°C. MBC value was defined as the lowest concentration preventing bacterial growth. The tests were done in triplicate.

Biofilm assay

Biofilm was performed by following the method from Mohamad (Hanafiah *et al.*, 2015) with modification. Biofilm formation assay was done by crystal-violet assay (Sigma-Aldrich, Missouri, USA) with a 96-well plate. A total of 1 mL *S. mutans* have been moved to 10 mL BHIB and been incubated at 37°C for 24 hours. Later, the culture is diluted 1:100 in BHIB and 150 μL of the bacteria been added into 96-well plate followed by 50 μL of the extracts (0.24 to 30 mg/mL) in the same well. NaF (1 mg/mL) and 10% DMSO acted as positive and negative control, respectively.

Then, the microplate well was incubated at 37°C for 24 hours. Next, the biofilm formation was quantified by measuring the absorbance of the solutions (biofilm and crystal violet) at 595 nm using a microplate reader (Model 680, Bio-Rad, California, USA).

Data analysis

All the data obtained had been analyzed by using Statistical Package for the Social Sciences (SPSS). In the disc diffusion assays, the data were analyzed by using two-way ANOVA as presents of two groups of independent variables which are the four different solvents in five different concentrations. Next, the anti-adherence and anti-biofilm test were analyzed by one-way ANOVA. Later, post hoc test was performed by using Turkey analysis for both data.

RESULTS AND DISCUSSION

Disc diffusion assay

Disc diffusion assay of leaves and flower extracts are shown in Tables 1 and 2, respectively. In this experiment, the antibacterial activity was calculated by measuring the inhibition zone that exhibits from each extract by using a metal ruler.

As seen in Table 1, all the values are represented as mean $\pm$ standard deviation. Values on the same row with a different (a, b, c) superscript letter were different significantly with $p<0.05$. Next, the values on the same column (*, **, ***) also changed with a significant level, $p<0.05$. The DMSO acted as a negative control, while NaF (1 mg/mL) represented as a positive control. From Table 1, it can be concluded that based on these four solvents, n-hexane and methanol extracts exhibit antibacterial properties as it increased the zone of inhibition against *S. mutans* on the agar.

Meanwhile, the DCM and acetone extracts did not show any antibacterial properties compared it inhibition zone with the negative control. Next, the data shows that the hexane and methanol extract inhibition zone increased with concentration-dependent manner.

Based on the results in Table 2, we can conclude that from all four solvents tested, only *n*-hexane and DCM extracts exhibit antibacterial properties as it increased the zone of inhibition against *S. mutans* on the agar. All the values represented as mean $\pm$ standard deviation. Values on the same row with a different (a, b, c, d) superscript letter were different significantly with $p<0.05$. Next, the values on the same column (*, **, ***) were also different from the significant level, $p<0.05$. Meanwhile, the methanol and acetone extracts do not show any inhibition zone. Next, the data indicate that upon an increment of n-hexane and DCM extracts concentration had increased the inhibition zone.

Four solvents were chosen based on their polarity

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level. N-hexane and DCM were used to extract non-polar compounds; meanwhile, acetone was used to remove semi-polar compounds, and methanol was used to extract polar compounds. Polar solvents have been used to isolate bioactive compounds that exhibited antibacterial and antiseptic properties such as phenol group (Joffry *et al.*, 2011). However, in this study, *n*-hexane and methanol leaves extract was found to be the most efficient to inhibit *S. mutans* growth. As for the flowers extract *n*-hexane and DCM solvents exhibited more antibacterial activity compared to the acetone and methanol.

As mentioned earlier, even though the polar compound extract generally showed the best effect as it can isolate most of the bioactive compounds in the plant. However, there are many reports stated that the antibacterial activity might not only depend on the solvent used but also depends on the compound structure in the extracts and the strain that been investigated (Padalia and Chanda, 2015).

Besides, (Truong *et al.*, 2019) stated that different organic solvents extracts have different phytochemical constituents in such different amounts, and this has led to differences in the impact of inhibition zone occurs. Therefore, *n*-hexane and methanol leaves extract as well as *n*-hexane, and DCM flowers extracts had been chosen to further analyze their antimicrobial activity in this study.

Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC)

Table 3 shows the MIC and MBC values of *n*-hexane, and methanol leaves extracts and *n*-hexane and DCM flowers extracts. The samples have been chosen in MIC and MBC analysis because they exhibited antibacterial activities in disc diffusion assay. The MIC value is the lowest concentration that inhibits the bacteria. In the MTT assay, the colour of formazan indicates that the bacteria are living. Meanwhile, the yellow colour indicates that the bacteria are inhibited when it exposed to the extract.

Hence, the MIC values for *n*-hexane and methanol leaves extracts were 25.0 mg/mL, respectively. In the meantime, MIC values for *n*-hexane and DCM flowers extracts were 12. 5 mg/mL, respectively. MBC value for *n*-hexane and methanol leaves extract were 50 and 100 mg/mL, respectively. Meanwhile, MBC values for n-hexane and DCM flowers extract were 50 mg/mL, respectively. MIC and MBC values for *n*-hexane were 1 and 2 mg/m, respectively.

Antibacterial agents were observed as bactericidal if MBC value was less than four times of MIC value. Meanwhile, bacteriostatic activity is defined when MBC values were more than four-times of MIC value (Clinical and Institute, 1999). In this study, the MBC value of *n*-hexane and DCM flowers extracts (50 mg/mL) was not more than four times of MIC value (12.5 mg/mL). MBC values of *n*-hexane and methanol leave also extract less than four-time of the MIC value. Therefore, samples from flowers extracts and leaves extracts were presumed as a bactericidal agent against *S. mutans*. (Varghese *et al.*, 2014) had suggested that *A. Paniculata* can be further studied for the production of new antibiotics as they have successfully proven that the crude extracts of this plant have potent antibacterial activity against pathogens.

Anti-biofilm assay

Reduction pattern in biofilm formation was a concentration-dependent manner to all samples tested (Figure 1). More concentration of samples was reducing more biofilm formation of *S. mutans*. Samples from flowers extracts (*n*-hexane and DCM) more potent than the leaves extract. About 60% of biofilm was inhibited from forming when treated with 50 mg/mL of flowers extract. At 100 mg/mL, biofilm formation was reduced to 70% of flowers extract compared than leaves extract. Meanwhile, NaF reduced the biofilm formation up to 84% at 1 mg/mL.

The effect of all samples towards *S. mutans* biofilm formation activities were concentration dependent-manner. The highest concentration reduced more biofilm formation activity. Meanwhile, the lowest concentration decreased less biofilm formation activity. This indicated that higher concentration comprised more bioactive compounds as compared to lower samples concentration. Previous studies reported that high concentration extracts increased their antibacterial (Ong *et al.*, 2011; Yoshida and Kuramitsu, 2002). This proved that extract concentration is also an essential element in determining the antibacterial activity of various plants.

CONCLUSIONS

In conclusion, *n*-hexane leaves extract, methanol leaves extract, *n*-hexane flowers extract, and DCM flowers extract of *A. paniculata* demonstrated bactericidal properties and exhibited anti-biofilm activities against *S. mutans*. All the antibacterial tests (Disc Diffusion assay, MIC and MBC) and anti-biofilm effects were concentration-dependent manners. In increasing of samples, concentration makes all the results higher and better.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Chandad, F., Grenier, D. 2007. Risk of bacterial resistance associated with systemic antibiotic therapy in periodontology. *Journal (Canadian Dental Association)*, 73:721–726.

CLSI 1999. *Methods for determining bactericidal activity of antimicrobial agents; approved guideline. Document M26-A*. Wayne, PA.

Das, K., Tiwari, R., Shrivastava, D. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of medicinal plants research*, 4:104–111.

Estari, M., Gujjeti, R. P. 2013. Phytochemical and antimicrobial activity of Acmella paniculata plant extracts. *Journal of Bio.Innovation*, 2:17–22.

Hanafiah, R. M., Aqma, W. S., Yaacob, W. A., Said, Z., Ibrahim, N. 2015. Antibacterial and biofilm inhibition activities of Melastoma malabathricum stem bark extract against Streptococcus mutants. *Malaysian Journal of Microbiology*, 11:199–206.

Joffry, S., Yob, N. J., Rofiee, M., Redzuan, M., Othman, F., Akim, A., Desa, M. N. M., Suhaili, Z., Zakaria, Z. A. 2011.

Liao, Y., Brandt, B. W., Li, J., Crielaard, W., Loveren, C. V., Deng, D. M. 2017. Fluoride resistance in Streptococcus mutans: a mini review. *Journal of Oral Microbiology*, 9(1):1344509–1344509.

Nomura, E. C. O., Rodrigues, M. R. A., Silva, C. F. D., Hamm, L. A., Nascimento, A. M., Souza, L. M. D., Cipriani, T. R., Baggio, C. H., Werner, M. F. D. P. 2013. Antinociceptive effects of ethanolic extract from the flowers of Acmella oleracea (L.) R.K. Jansen in mice. *Journal of Ethnopharmacology*, 150:583–589.

Ong, H. M., Mohamad, A. S., Makhtar, N. A., Khalid, M. H., Khalid, S., Perimal, E. K., Mastuki, S. N., Zakaria, Z. A., Lajis, N., Israf, D. A., Sulaiman, M. R. 2011. Antinociceptive activity of methanolic extract of Acmella uliginosa (Sw.) Cass. *Journal of Ethnopharmacology*, 133(1):227–233.

Padalia, H., Chanda, S. 2015. Antimicrobial Efficacy of Different Solvent Extracts of Tagetes erecta L. Flower, Alone and in Combination with Antibiotics. *Applied Microbiology: open access*, 1(1).

Shai, L. J., McGaw, L. J., Aderogba, M. A., Mdee, L. K., Eloff, J. N. 2008. Four pentacyclic triterpenoids with antifungal and antibacterial activity from Curtisia dentata (Burm.f) C.A. Sm. leaves. *Journal of Ethnopharmacology*, 119(2):238–244.

Teles, R. P., Teles, F. R. F. 2009. Antimicrobial agents used in the control of periodontal biofilms: effective adjuncts to mechanical plaque control?

Truong, D.-H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., Nguyen, H. C. 2019. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. *Journal of Food Quality*, 2019:1–9.

Varghese, P. K., Mohan, R., Abdulla, M., H, M. 2014. Antibacterial activity of Acmella paniculata extract on human pathogenic bacteria. *European Journal of Herbal Medicine*, 132:132–134.

Yoshida, A., Kuramitsu, H. K. 2002. Multiple Streptococcus mutans Genes Are Involved in Biofilm Formation. *Applied and Environmental Microbiology*, 68(12):6283–6291.