Antimicrobial, antioxidant, anti-inflammatory activities and toxicity of semi-purified *Anacardium occidentale* leaf extract

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**ABSTRACT**

Periodontal diseases, the most prevalence of inflammatory oral diseases in adulthood, are caused by the imbalance of host-bacterial interaction. Many parts of cashew (*Anacardium occidentale*) plant have been used traditionally for therapeutic purposes such as to treat diarrhea, thrush, ulcers, toothache, gum problem and malaria. The crude extract of cashew leaves has been shown to have antimicrobial, antioxidant and anti-inflammatory properties. The aims of this *in vitro* study were to investigate the antimicrobial, antioxidant, anti-inflammatory activities and toxicity of semi-purified *A. occidentale* leaf extract. Using the Sephadex LH-20 column, the five semi-purified *A. occidentale* fractions were isolated (Fraction A to E), and screened for antimicrobial activity against *Porphyromonas gingivalis* using agar diffusion method. Two fractions that gave the widest clear zones, were further determined for minimal inhibitory concentration (MIC) using microdilution method, and their free radical scavenging properties were tested by DPPH free radical. The anti-inflammatory activity was investigated with 5 and 10 µg/ml LPS treated human gingival connective tissue fibroblasts (HGFs) at MIC, 2 and 4 times of MIC of the two fractions. PGE2 levels were determined using ELISA. HGFs toxicity was investigated using MTT colorimetric assay. In this study, semi-purified *A. occidentale* leaf extract fraction A and C presented the widest clear zones and their MIC were 1.25 and 2.5 mg/ml, respectively. The free radical scavenging activity (IC50 value) of fraction C was significant stronger than ascorbic acid (control) and fraction A at 1.29 ± 0.03, 2.20 ± 0.04, and 16.88 ± 0.51 µg/ml, respectively. Both fractions, at their MIC, 2 and 4 times of MIC, presented PGE2 levels lower than the controls significantly at p < 0.05. The cell survival rate of HGFs with fraction A was higher than the controls and fraction C, respectively. This study showed that the fraction A and C semi-purified *A. occidentale* leaf extracts have antimicrobial, antioxidant and anti-inflammatory activities. The extract fraction A is shown non-toxicity to HGFs. Therefore, the fraction A semi-purified *A. occidentale* leaf extract seems to have potential to use for alternative medicine in the prevention and/or treatment of inflammatory oral diseases such as gingivitis and periodontitis.

**Key words:** *A. occidentale*, antimicrobial, *P. gingivalis*, antioxidant, anti-inflammatory, PGE2

**INTRODUCTION**

Periodontal diseases, the most prevalence of inflammatory oral diseases in adulthood (World Health Organization, 2003; Frencken et al., 2017), are caused by ecological shift of bacteria in dental biofilm that stimulates the host immune response which can lead to loss of teeth (Johansson and Dahlen, 2018). The inflammatory processes start and locate at gingival epithelium and connective tissues known as gingivitis, if these processes are not stopped or received proper clearance, the pathogenesis continues and extends deep into the supporting periodontal ligament and alveolar bone, the tissue destruction is called periodontitis. Periodontitis has been shown to relate to
specific gram-negative anaerobic bacteria such as Porphyromonas gingivalis (Mysak et al., 2014; How et al., 2016) which was found in subgingival biofilm of patients with chronic periodontitis up to 85.75% (How et al., 2016). Lipopolysaccharide (LPS) of gram-negative bacteria, through the stimulation of toll-like receptor 4 (TLR4) (Matsuura, 2013), induces host cells to express pro-inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α (Graves, 2008) and subsequent prostaglandin E₂ (PGE₂) production (Bage et al., 2011). PGE₂ is well known to play the important role in inflammatory responses and alveolar bone resorption (Asam, 2011). Patients with periodontitis have a high PGE₂ level and the level will be decreased after patients receive periodontal treatment (Kumar et al., 2013). Meanwhile, the initiation of innate defense against bacterial infection, upon phagocytic mechanism by neutrophils, causes oxidative stress, the imbalance between oxidant and antioxidant activities. The overproduction of reactive oxygen species (singlet oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide) leads to tissue destruction (Vincent et al., 2017). Previous studies reported that patients with periodontitis had reduced antioxidant capacity (Zhang, 2016) and after receiving periodontal treatment (Yang et al., 2014) or uptake antioxidant agents such as ascorbic acid (Shimabukuro et al., 2015), alphatocopherol (vitamin E) (Hatipoglu et al., 2016), and coenzyme Q₁₀ (Sale et al., 2014), the patients had increased antioxidant levels (Thomas et al., 2014).

Anacardium occidentale, commonly known as cashew, is a tropical nut tree found to spread widely in South America, Asia and Africa. It is a member of genus Anacardium belonging to family Anacardiaceae (Dendena and Corsi, 2014). In traditional medicine, the leaves are used to treat tooth aches, gum problems and malaria. The bark is used to treat fevers, diabetes, detoxify snake bite to tooth aches, diabetes, detoxify snake bite. In traditional medicine, the methanol dissolved leaf extract of A. occidentale is used to treat diarrhea, thrush, ulcers (Odo, 2017), diabetes mellitus (Ruby et al., 2007), and cancer (Maity et al., 2015) due to their antimicrobial activity against Periodontal pathogens. (Mustapha and Hafsat, 2007), antifungal (Kanan et al., 2009), anti-ulcer (Odo, 2017), antihyperglycemic (Ukwenny et al., 2012), and antimutagenic effects (Barcelos et al., 2007). The crude extract of cashew leaves was shown to have antioxidant and anti-inflammatory properties (Natalia et al., 2017). In addition, Srissawat et al. (2005) showed that the crude extract of A. occidentale leaves had antimicrobial activity against specific periodontopathic bacteria: P. gingivalis, Prevotella intermedia and Aggregatibacter actinomycetemcomitans. The extract provided a tendency to reduce PGE₂ and non-toxicity on human gingival fibroblasts. In the light of a wider understanding of this medicinal plant and for utilizing the extract for preventive and therapeutic use, the present study aimed to purify the crude extract, and then test for its properties.

**MATERIALS AND METHODS**

**Preparation of semi-purified A. occidentale leaf extract**

Cashew leaves, collected from Songkhla province in Southern part of Thailand, were washed and oven-dried at 50°C for 48 h. The ground leaves weight 100 g were macerated with ethanol 300 ml for 3 days. Filtrated supernatant was concentrated by rotary evaporation to obtain the crude extract 12.61 g. Using Sephadex LH-20 column (Amersham, 2002), the methanol dissolved-crude extract was eluted by methanol. All fractions were analyzed by thin-layer chromatography (Kumar et al., 2013) and pulled, according to the physical visibility, to give 5 main fractions (fraction A to E).

**Screening the antimicrobial activity against P. gingivalis and determination of minimal inhibitory concentration (MIC) of the semi-purified A. occidentale leaf extract**

Five fractions of the semi-purified A. occidentale leaf extract, each dissolved in 20% dimethylsulfoxide (DMSO), were screened for antimicrobial activity against P. gingivalis using agar diffusion method. The 500 µl (1 x 10⁸ CFU/ml) of P. gingivalis W80 were added to each blood agar plates supplemented with 5 µg/ml hemin and 0.5 µg/ml vitamin K. Each extract was placed into a 6 mm hole of agar plates and incubated at 37°C under anaerobic condition overnight. The solvent 20% DMSO was used as control. Two fractions that gave the widest inhibition zones were selected and further determined for their minimal inhibitory concentration (MIC) using microdilution method. The agar diffusion and microdilution experiments were done in separate triplicate tests. The data of clear zone width were expressed as the mean ± standard deviation (S.D.).

**Antioxidant activity**

The antioxidant activity of the semi-purified A. occidentale leaf extracts, fraction A and fraction C, was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. Briefly, DPPH solution (6x10⁻⁵ M) was incubated with an equal volume of fraction A (1-400 µg/ml) and fraction C (0.2-20 µg/ml) in absolute ethanol for 30 min in the dark at room temperature. The reduction in the DPPH radical was
measured using 96 well microplates (PowerWaveX, Biotek) spectrophotometric absorbance at 517 nm. Ascorbic acid was used as a positive control. The negative control was prepared as described above, but without the extract or ascorbic acid. All samples were tested in separate triplicate experiments. DPPH radical scavenging activity was presented in term of % inhibition, which was calculated as follows:

\[
\text{% inhibition} = \left(\frac{\text{AC} - \text{AS}}{\text{AC}}\right) \times 100
\]

Where AC is the absorbance of the control reaction (containing all reagents except the semi-purified *A. occidentale* leaf extract and ascorbic acid) and AS is the absorbance of the semi-purified *A. occidentale* leaf extract fraction A, fraction C and ascorbic acid. The effective concentration of sample required to scavenge DPPH by 50% (IC$_{50}$ value) was obtained by linear regression analysis of dose responds curve plotting between % inhibition and concentration. The IC$_{50}$ values were expressed as mean ± S.D.

**Human gingival connective tissue fibroblasts culture**

Human gingival connective tissue fibroblasts (HGFs) culture was performed using the method described in previous study with some modification. This experiment was approved by the Ethic committee, Faculty of Dentistry, Prince of Songkla University (REC project No. EC5505-22-L). Briefly, HGFs were derived from the explants of healthy gingiva from gingival surgery or gingival remnant from extracted sound tooth. Each biopsy was transported in sterile DMEM pH 7.2. The biopsy was washed extensively and cut into 1x1 mm/piece. Then it was placed in a plastic culture dishes in DMEM pH 7.2 containing deactivated 10% FBS and 100 µg/ml penicillin-streptomycin. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO$_2$ and 95% air. The media were replaced every 2 days until confluence, HGFs were split 1:3 and this was denoted as the 1$^{st}$ passage. Cells from the same passage, which were between their 5$^{th}$-10$^{th}$passage, were used for the experiments.

**Anti-inflammatory activity**

Semi-purified *A. occidentale* leaf extract fraction A and fraction C at their MIC, 2 times, and 4 times of MIC were evaluated for anti-inflammatory activities against PGE$_2$ on HGFs. Briefly, HGFs were added to each 24 well culture plates at 5 × 10$^4$ cells in 1 ml of DMEM supplemented with 2% FBS and antibiotics. They were incubated at 37°C in humidified atmosphere of 95% air and 5% CO$_2$ overnight. After that, HGFs were treated with 5 and 10 µg/ml LPS of *Escherichia coli* (Sigma®, USA) and further incubated for 24 h before adding the studied extracts. After overnight incubation, all of the experiment supernatants were collected. The positive controls of these experiments were LPS treated HGFs without the extracts and the negative control was LPS untreated HGFs without the extracts. All samples were tested in separate triplicate experiments. PGE$_2$ of the supernatant was measured by a commercial specific enzyme-linked immunoabsorbent assay (ELISA) kit (R&D system, USA). An ELISA reader (Ceres UV 900 HDi, Biotrak Instrument, USA) was used to measure the spectrophotometric absorbance at 450 nm (Kumar et al., 2013). The data were expressed as mean ± S.D.

**Cell viability/Cytotoxicity**

The cytotoxicity of HGFs was tested using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma®, USA). Briefly, MTT solution was prepared at 5 mg/ml in DMEM just before use and filtered through a 0.22 µm filter for sterilization. After removing the supernatant from anti-inflammatory experiment, all the well culture plates were replaced with MTT solution in DMEM. Cells were incubated for 4 h at 37°C. Thereafter, the medium was removed and the cells culture plates were washed twice with phosphate buffer saline (PBS). The DMSO solution was added to dissolve formazan crystals and mixed to ensure complete solubilization. The absorbance was read at 570 nm with a microplate reader. All samples were tested in separate triplicate experiments. The cells survival rate was calculated as follows:

\[
\text{Survival rate (}) = \left(\frac{A_c - A_n}{A_c - A_0}\right) \times 100
\]

Where $A_c$ is the absorbance of the test group reaction, $A_n$ is the absorbance of the negative control reaction and $A_0$ is the absorbance of the blank reaction. The data were expressed as mean ± S.D.

**Statistical analysis**

The mean values of all experiments (except MIC) were compared using a one-way analysis of variance (ANOVA). The $p$-values were considered significant when $p < 0.05$. The MIC was analyzed by descriptive statistic.

**RESULTS**

**Screening the antibacterial activity against *P. gingivalis* and determination of minimal inhibitory concentration (MIC) of the extract**

The results from agar diffusion method showed that semi-purified *A. occidentale* leaf extract fraction A presented the widest zones of inhibition against *P. gingivalis*. The mean values of three repetitions, analyzed by one-way ANOVA, showed significantly difference among the inhibition zones of each fraction of semi-purified *A. occidentale* leaf extract at $p < 0.05$. The inhibition zones of semi-purified *A.
Table 1: Diameters of the zones of bacterial growth inhibition against *P. gingivalis* of semi-purified *A. occidentale* leaf extract fraction A to E.

<table>
<thead>
<tr>
<th>Fractions of <em>A. occidentale</em> leaf extract</th>
<th>Zones of inhibition (cm.) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.72 ± 0.03*</td>
</tr>
<tr>
<td>B</td>
<td>1.02 ± 0.08*</td>
</tr>
<tr>
<td>C</td>
<td>1.95 ± 0.05*</td>
</tr>
<tr>
<td>D</td>
<td>1.33 ± 0.03*</td>
</tr>
<tr>
<td>E</td>
<td>1.58 ± 0.03*</td>
</tr>
<tr>
<td>20% DMSO</td>
<td>0.6 ± 0.00</td>
</tr>
</tbody>
</table>

The values are expressed as means of 3 repetitions and standard deviations (S.D.).

* Means significantly different between each other fraction at *p* < 0.05, analyzed by one-way ANOVA.

Table 2: IC<sub>50</sub> value of ascorbic acid, semi-purified *A. occidentale* leaf extract fraction A, and fraction C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; value ± S.D. (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>2.20 ± 0.04*</td>
</tr>
<tr>
<td>Fraction A</td>
<td>16.88 ± 0.51*</td>
</tr>
<tr>
<td>Fraction C</td>
<td>1.29 ± 0.03*</td>
</tr>
</tbody>
</table>

The values are expressed as means of 3 repetitions and standard deviations (S.D.).

* means significantly different between each other sample at *p* < 0.05, analyzed by one-way ANOVA.

*occidentale* leaf extract are shown in Table 1. Fractions A and C that gave the widest inhibition zones, were subsequently determined for their minimal inhibitory concentrations (MIC) using microdilution method. The results from three repetitions showed their MIC at 1.25 and 2.5 mg/ml, respectively.

Antioxidant activity (DPPH free radical scavenging activity)

The results of antioxidant activity of semi-purified *A. occidentale* leaf extract fraction A, fraction C and ascorbic acid showed that fraction C provided the highest potential, while fraction A provided the lowest potential. The mean values of three repetitions, analyzed by one-way ANOVA, showed significantly different at *p* < 0.05. IC<sub>50</sub> values of ascorbic acid, semi-purified *A. occidentale* leaf extract fraction A and fraction C are shown in Table 2.

Anti-inflammatory activity

After 24 h stimulation, the 5 and 10 µg/ml LPS treated HGFs (positive controls) showed statistically difference in PGE<sub>2</sub> expression when compared with LPS untreated HGFs (negative control). However, there was no dose dependence of LPS stimulation in term of PGE<sub>2</sub> expression. After 24 h incubation of LPS treated cells with the tests, the results showed that semi-purified *A. occidentale* leaf extract fraction A and C contained anti-inflammatory activities, which demonstrated by PGE<sub>2</sub> reduction, in all studied concentrations (one way ANOVA, significantly different at *p* < 0.05). At the same stimulated level of PGE<sub>2</sub>, the fraction A seemed to have better anti-inflammatory activities than the fraction C both at 2.5 and 5 mg/ml; however, there was no significant difference at *p* < 0.05. The anti-inflammatory activities, which were demonstrated by PGE<sub>2</sub> level, of semi-purified *A. occidentale* leaf extracts to LPS treated and untreated HGFs are shown in Figures 1 and 2.

Cell viability/cytotoxicity

The results of the MTT assay showed that 1.25 and 2.5 mg/ml, but not 5 mg/ml of the semi-purified *A. occidentale* leaf extract fraction A presented a greater survival rate of HGFs than the controls significantly at *p* < 0.05. While 2.5 and 5 mg/ml of semi-purified *A. occidentale* leaf extract fraction C presented cell survival rate lower than the controls significantly at *p* < 0.05, but at 10 mg/ml was not
Figure 1: The anti-inflammatory activity graph shows PGE2 expression of unstimulated HGFs (DMEM; negative control), stimulated with 5 µg/ml and 10 µg/ml LPS without the fraction A extract (positive controls), 5 and 10 µg/ml LPS with the fraction A extract at MIC, 2 times, and 4 times of MIC. The values are expressed as means of 3 repetitions and standard deviations (S.D.).

a Compared to the negative control group significantly different at p < 0.05.

b Compared to 5 µg/ml LPS treated HGFs without the extract significantly different at p < 0.05.

c Compared to 10 µg/ml LPS treated HGFs without the extract significantly different at p < 0.05.

significantly different. The results of 1.5625, 6.25 and 12.5% DMSO, which were at the same concentrations used to dissolve the tested extracts, also showed no significant difference in the cell survival rate when compared with the controls at p < 0.05. The cell viability of HGFs with the tested extracts and DMSO is shown in Figure 3.

**DISCUSSION**

Currently, people are widely interested in the use of natural products, especially parts and products of plants, in a variety of medicinal, pharmaceutical, nutraceutical and cosmetic applications. Among these, many medicinal plants have been used in several forms to control local etiologic factors and in the treatment of periodontal diseases. For example, dentifrice containing *Acacia catechu* and mouth rinse containing *Aloe vera* had effects on reducing dental biofilm and gingivitis (Kala et al., 2015; Vangipuram et al., 2016), topical gel containing tea tree oil reduced gingival inflammation (Soukoulis and Hirsch, 2004), as well as mouth rinse containing pomegranate and chamomile extracts were shown to reduce gingival bleeding in patients with gingivitis (Batista et al., 2014). Our serial studies on mouthwash and dentifrice containing *A. occidentale* leaf extract and *Punica granatum* pericarp also demonstrated the reduction in dental biofilm deposition and gingivitis (manuscript in preparation). For standardization and understanding that could enhance their uses, the present study includes detailed analysis of semi-purification *A. occidentale* leaf extract properties.

Srisawat (2007) studied the antimicrobial, anti-inflammatory activities and toxicity of the crude extract of some medicinal plants (*A. occidentale* leaves and bark,
**Figure 2**: The anti-inflammatory activity graph shows PGE$_2$ expression of unstimulated HGFs (DMEM; negative control), stimulated with 5 µg/ml and 10 µg/ml LPS without the fraction C extract (positive controls), 5 and 10 µg/ml LPS with the fraction C extract at MIC, 2 times, and 4 times of MIC. The values are expressed as means of 3 repetitions and standard deviations (S.D.).

*Compared to the negative control group significantly different at $p < 0.05$.

*Compared to 5 µg/ml LPS treated HGFs without the extract significantly different at $p < 0.05$.

*Compared to 10 µg/ml LPS treated HGFs without the extract significantly different at $p < 0.05$.

**Syzygium cumini** leaves and bark, **Punica granatum** pericarp, and **Rhinacanthus nasutus** leaves. The study reported that **A. occidentale** leaf and bark extracts presented better antimicrobial properties against *P. gingivalis* at 1.56 and 0.48 mg/ml respectively than the other studied plants; however, **A. occidentale** bark showed toxicity to HGFs. Numerous previous studies on Anacardiaceae family also support the fact that the plant antimicrobial activities of many parts of **Anacardium excelsum** (leaves, integument, flowers, seed and seed coat) showed their antimicrobial activities against gram positive bacteria such as *Basilus subtilis* (Celis et al., 2011), and also, **Semicarpus anacardium** nut extract had antimicrobial activity against gram positive and gram negative bacteria including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively (Mohanta et al., 2007). Most plants contain phenolic compounds, which the hydroxyl groups on phenolic ring are related to microorganism toxicity by increasing hydroxylation (Ojezele and Agunbiade, 2013; Hintz et al., 2015). The leaves of members of the Anacardiaceae family such as **A. occidentale**, similar to other plants, contain polyphenols, tannin, alkaloid and saponin (Ojezele and Agunbiade, 2013). The antimicrobial activity of **A. occidentale** crude extract in the previous study and also in this study, therefore, may be attributed to these compositions. The semi-purified **A. occidentale** leaf extract fraction A was also shown the MIC (1.25 mg/ml) against *P. gingivalis* lower than that of the crude extract from the previous study (1.56 mg/ml) (Srisawat, 2007). Based results of using Sephadex LH-20 column, we speculate that phenolic compounds in fraction A which contain the higher molecular weight than that in the fraction C (MIC 2.5 mg/ml) provides better antimicrobial property. This point of view is supported by the study of antimicrobial activities against *S. aureus, P. aeruginosa* and *Enterococcus faecalis* of tannin-rich fractions from **Anacardium humile**.
Figure 3: Cells viability graph shows cell survival rate (%) of DMEM (control), DMSO at the same concentration used to dissolve the fraction A and fraction C extracts, the fraction A and fraction C extract at MIC, 2 times, and 4 times of MIC. The values are expressed as means of 3 repetitions and standard deviations (S.D.). *Compared to the control significantly different at $p < 0.05$.

(Anacardiaceae family, found in the Brazilian Savanna) leaf extract (Ferreira et al., 2012). These properties of tannins are based on their chemical structures having two or three phenolic hydroxyl groups on a phenyl ring, in a molecule of moderately large size.

Phenolic compounds contain ability to scavenge free radicals. Flavonoids are the large group of phenolic compounds; act as antioxidant including suppression of ROS formation, inhibition of enzymes involved in ROS generation and lipid oxidation (Kumar and Pandey, 2013). Junior et al. (2007) showed that leaves of A. occidentale contained total phenolic and exhibited free radical scavenging activity similar to butylated hydroxytoluene (BHT), the synthetic antioxidant. Several studies have reported the Anacardiaceae family, such as Lannea barteri stem bark and root extracts and Semecarpus anacardium leaf extract which contained polyphenols, flavonoids, and tannins, presented free radical scavenging activity similar to ascorbic acid (Koné et al., 2011; Sheikh et al., 2016; Onuh et al., 2017). The free radical scavenging activity of semi-purified extracts in the present study, therefore, may due to the presence of phenolic, tannins, and flavonoids. It is of interest to note that semi-purified extract fraction C, with lower molecular weight than fraction A, provided stronger potential in free radical scavenging activity than ascorbic acid and fraction A in the present study. The antioxidant activity of compounds is due mainly to their redox properties, which allow them to act as reducing agents or hydrogen atom donors and that relate to the number and position, o -or p –position, of hydroxyl groups in the molecule (Castellano et al., 2012).

Flavonoids are able to inhibit the expression of mediators in inflammatory processes such as cytokines, chemokines, and prostaglandins (Kumar and Pandey, 2013). Also, saponins may involve in the inhibition of inflammatory mediators such as histamine, serotonin, and prostaglandins (Desai et al., 2009). Souzo et al. (2017) investigated the anti-inflammatory property of A. occidentale leaf extract in macrophage cells RAW 264.7. The study found that the extract was able to inhibit the release of pro-inflammatory cytokines (IL-1β, TNF-α) induced by 1 µg/ml LPS stimulated macrophage cells RAW 264.7. Srisawat (2007) investigated the anti-inflammatory activity of A. occidentale leaf extract in HGFs treated with 1 µg/ml LPS. The study
found that the extract had a tendency to reduce PGE₂. It was observed that all of the studied concentration of *A. occidentale* semi-purified leaf extracts fractions A and C had PGE₂ levels lower than the positive controls with significant difference at *p* < 0.05, which demonstrated their anti-inflammatory activities. The anti-inflammatory property of the semi-purified *A. occidentale* leaf extract was better than the crude extract.

The investigation of cells viability showed that all concentrations of the studied semi-purified *A. occidentale* leaf extracts, except at 2.5, 5 mg/ml of fraction C, had no effects on cell death. Meanwhile, the studied concentrations of DMSO, which was the same concentration used to dissolve each extract, also showed no effects on cell viability. This means that toxicity of semi-purified fraction C, therefore, should be accounted by the extract itself. This point of view emphasizes the argument that isolated or synthesized active compounds may be toxic to human. On the other hand, the previous study of crude *A. occidentale* leaf extract showed the cell survival as high as 156.25 mg/ml for all time-point studied (12, 24, and 48 h) (Srisawat, 2007). This may imply that *A. occidentale* crude leaf extract is quite safe and in this study, at least, at 4 times MIC of the semi-purified fraction A.

Taken together, the semi-purified *A. occidentale* leaf extracts fraction A and C showed antimicrobial property against *P. gingivalis* and had anti-inflammatory activities in term of PGE₂ reduction. The semi-purified *A. occidentale* leaf extract fraction A had no toxic to HGFs but provided weak antioxidant property. On the contrary, the semi-purified *A. occidentale* leaf extract fraction C provided strong antioxidant property, but had toxicity to HGFs. Therefore, the semi-purified *A. occidentale* leaf extract fraction A has the potential to be used as an alternative medicine for the treatment of infectious-inflammatory oral diseases. Within the limitation of the study, there is concern that there are unaccounted factors, such as growing environment, growing season, climate, temperature, light, soil type, and solvent used which could affect phenolic contents in plants and contribute to their properties.

**CONCLUSION**

This study shows that the fractions A and C semi-purified *A. occidentale* leaf extracts have antimicrobial, antioxidant and anti-inflammatory activities. The extract fraction A showed non-toxicity to HGFs. Therefore, the fraction A semi-purified *A. occidentale* leaf extract seems to have potential to be used as alternative medicine in the prevention and/or treatment of infectious-inflammatory diseases such as gingivitis and periodontitis.

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