

Antioxidant and anti-inflammatory activities of macerated herbal oil in dental pulp cells

Fahsai Kantawong ^{1*} Sutinee Mungkala ¹ Sawinee Tamang ¹ Ruthairat Manaphan ¹
Pichaporn Thaweean ¹ Phenphichar Wanachantararak ²

¹Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand

²The Dental Research Center, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand

ARTICLE INFO

Article history:

Received 31 May 2018

Accepted as revised 21 August 2018

Available online 29 August 2018

Keywords:

Macerated herbal oil, Dental Pulp Cells,
Anti-inflammation, Anti-Oxidant

ABSTRACT

Background: Sesame oil was used as an oil-pulling mouthwash to reduce the microorganisms that cause gum diseases. Herbs can be used in combination with sesame oil in oral health care. The previous study showed that macerated herbal oil decreased inflammation in human mesenchymal stem cells (HMSCs). It could be hypothesized that the addition of herbs should improve the efficiency in oil pulling.

Objectives: This study aimed to improve the efficiency of sesame oil for oral healthcare and to compare the total phenolic contents and antioxidant activities between sesame oil and macerated herbal oil. Moreover, this study compared the anti-inflammation activities between sesame oil and macerated herbal oil on lipopolysaccharide (LPS)-induced dental pulp cells (DPCs).

Materials and methods: The amount of total phenolic content was determined using Folin-Ciocalteu reagent. Antioxidant activity was investigated using DPPH assay and ferric-reducing power assay. MTT assay was used to determine cytotoxic effect and gene expression was studied using real-time PCR technique.

Results: The phenolic concentration in sesame and macerated herbal oils were not significantly different. However, antioxidant assays indicated that the antioxidant activity of macerated herbal oil was significantly higher than that of sesame oil. Both oils showed no cytotoxic effect on DPCs. Both oils also induced cell proliferation and increased expression of the thioredoxin (TXN) gene. Inflammation of DPCs were induced by LPS and treated with herbal oil or sesame oil to study cyclooxygenase-2 (COX-2) and interleukin-6 (IL-6) gene expression. The results of real-time PCR showed a decrease of COX-2 and IL-6 gene expression when LPS-induced DPCs were treated with macerated herbal oil compared to sesame oil.

Conclusion: Macerated herbal oil presented many benefits over sesame oil, including anti-oxidation and anti-inflammation activities. Therefore, adding herbs to sesame oil for oil pulling might protect against periodontal disease.

Introduction

Oil pulling has been used extensively as a traditional Indian folk remedy to prevent tooth decay, oral malodor, and bleeding gums.^{1, 2} Asian people have been gargling

vegetable oil as a mouthwash for thousands of years. It is believed that when the oil is moving in the mouth, it will pull out microorganisms from the teeth, gums and tongue. Oils commonly used in oil pulling include sesame, coconut and sunflower oils.

Peedikayil et al. found that coconut oil pulling could be an effective adjuvant procedure in decreasing plaque formation and plaque induced gingivitis.³ Sesame has been shown to help relieve infections by reducing *Streptococcus mutans* in plaque and saliva.² Some studies have demonstrated

* Corresponding author.

Author's Address: Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand

** E-mail address: fahsai.k@cmu.ac.th

doi: 10.14456/jams.2018.22

E-ISSN: 2539-6056

that the efficacies of sesame oil and chlorhexidine mouthwash were equivalent. For example, Asokan et al. reported that sesame oil pulling was as effective as chlorhexidine mouthwash against halitosis and microorganisms.⁴ Sood et al. demonstrated that sesame oil pulling was as effective as chlorhexidine in reducing oral malodor and the microbes that cause it.⁵ Sesame seeds mainly contain oleic and linoleic acid⁶; these substances act as anti-inflammatories by reducing IL-6 and COX-2 expression.⁷ COX-2 and IL-6 were considered as inflammatory enzymes and inflammatory cytokines, respectively.^{8,9} Given these properties, sesame oil can be used as an oil-pulling mouthwash to reduce the microorganisms that cause gum disease.

Herbs can be used in combination with sesame oil in oral health care.¹⁰⁻¹² Many of the herbs used in this research, including *Zingiber montanum*, *Zingiber zerumbet*, *Plantago major* Linn, *Amomum biflorum* Jack, and the peels and leaves of *Citrus hystrix*, have been shown to have medicinal properties.¹³⁻¹⁷ Previous study indicated that the rhizome of *Zingiber montanum* possessed antioxidant and anti-inflammatory activities.¹³ Recently, Verma et al. demonstrated the antimicrobial activities of *Zingiber montanum* essential oil against bacteria and fungi.¹⁸ *Zerumbone* from *Zingiber zerumbet* was reported to reduce the inflammatory response of acute lung injury via expression of ICAM-1, IL-1 β , and MIP-2.¹⁹ *Plantago major* extract protected renal tissue against doxorubicin-induced renal inflammation.²⁰ Hexane extract of the peels of *Citrus hystrix* fruits showed cytotoxicity against human pancreatic cancer cells.²¹

These herbs were simmered in hot sesame oil; a method similar to the maceration method that has been applied to vegetable oils and herbs since ancient times.²² Our previous study showed that macerated herbal oil decreased inflammation in human mesenchymal stem cells (HMSCs).²³ This study tried to modify the macerated herbal oil for dental care purpose and hypothesized that herbs, in addition to their various medicinal properties, could increase the oil-pulling efficiency of sesame oil. The aims of this study are to: 1) compare the total phenolic content and the antioxidant and anti-inflammatory activities of sesame oil against macerated herbal oil and 2) study the toxicity of sesame oil and macerated herbal oil on DPCs.

Materials and methods

Preparation of macerated herbal oil

The preparation of macerated herbal oil was modified from the previous study.²³ All the fresh herbs were washed and sliced. A volume of 750 ml of cold-pressed sesame oil (purchased from a local manufacturer) was heated with 250 g of crushed cockle shell on a charcoal stove for 10 min. 100 g of *Zingiber cassumunar*, 160 g of *Zingiber zerumbet*, 200 g of *Plantago major* Linn, 150 g of *Citrus hystrix* peels, 70 g of *Citrus hystrix* leaves, and 100 g of *Amomum biflorum* were added into the heated sesame oil. All the components were slowly stirred for 1.5 h. The oil was left overnight, then centrifuged at 2,000 rpm for 15 min. The herb residual was precipitated and the oil was separated into a new container. Clear yellow oil with the soft aroma

of the herbs was aliquoted in closed containers before storing at 4 °C until use. The previous recipe and the modified recipe were shown in Table 1.

Table 1 Previous recipe²³ and the modified recipe.

Components	Weight percent (wt%) Previous study	Weight percent (wt%) Present study
Sesame oil	47	40
Crushed cockle shells	10	14
<i>Zingiber cassumunar</i> Roxb.	13	6
<i>Zingiber zerumbet</i>	7	9
<i>Plantago major</i> Linn	2	12
Peels of <i>Citrus hystrix</i>	7	9
Leaves of <i>Citrus hystrix</i>	2	4
<i>Amomum biflorum</i>	3	6
Menthol	5	-
Camphor	2	-
Borneol	2	-
Total	100	100

Total phenolic content

The total phenolic content was performed as described in the previous study.²³ The sesame oil and macerated herbal oil were diluted with dimethyl sulfoxide (DMSO) to the dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128. Thereafter, 50 μ l of each dilution was mixed with 2.5 ml Folin Ciocalteu reagent, and 2.0 ml Na₂CO₃ was added and mixed, before incubating at 45°C for 15 min. A blue color was observed, and the absorbance was measured at a wavelength of 764 nm. The mixture of 2.5 ml Folin Ciocalteu reagent and 2.0 ml Na₂CO₃ was used as blank for the zero setting. A standard calibration curve was prepared using different concentrations of gallic acid in DMSO. Three replicate assays were performed for each dilution, and the total phenolic concentrations were calculated from the standard curve and reported as mg/ml equivalent to standard gallic acid.

Antioxidant activity

The antioxidant activities were performed as described in the previous study²³.

(1) DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging Assay

The sesame oil and macerated herbal oil were diluted with DMSO to the dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128. Then, 50 μ l of each dilution was mixed with 2,950 μ l of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. The mixture was allowed to react in the dark at room temperature for 15 min. The absorbance was measured at a wavelength of 515 nm using distilled water to set zero. Standard trolox was used as the positive control. DMSO solution of volume 2,950 μ l plus 50 μ l of each dilution was used as a sample blank. DPPH solution of volume 2,950 μ l plus 50 μ l DMSO was used as a control for calculation. Three replicate assays were performed for each dilution, and the %DPPH inhibition was calculated from the following

equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance Blank} - \text{Absorbance Sample}}{\text{Absorbance Blank}} \times 100$$

(2) Ferric-reducing power activit

The ferric-reducing power activity was performed as described in the previous study.²³ The sesame oil and macerated herbal oil were diluted with DMSO to the dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128. For reducing power, 2.5 µl of each dilution of sesame oil or macerated herbal oil was mixed with 2.5 ml PBS. Then 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$) was added to the mixture and incubated at 50 °C for 20 min. After the reaction cooled down, 2.5 ml of 10% trichloroacetic acid was added and mixed. The reaction was centrifuged at 3,000 rpm for 10 min. A volume of 2.5 ml of the supernatant was separated and mixed with 2.5 ml of deionized water. A volume of 0.5 ml of 0.1% ferric chloride was added to the reaction and allowed to stand for 10 min. The absorbance was measured at 700 nm. Three replicate assays were performed for the statistical analysis. Various concentrations of butylated hydroxytoluene (BHT) in DMSO were used to generate a standard curve. The reducing power was calculated from the standard curve and reported as reducing power equivalent to BHT.

DPCs isolation

Ethical approval by the Ethics Committee of the Faculty of Dentistry, Chiang Mai University (40/2559) was obtained for this study. DPCs were prepared as described previously by Fahsai et al.²⁴ Briefly, the gum tissue was removed with a scalpel blade No. 15, and the teeth were wiped with povidone-iodine for 2 min, then wiped with 70% ethanol for 2 min to remove bacteria. The teeth were notched at a dentogingival junction with a chisel before immersion in 15 ml plastic tubes containing complete Dulbecco Modified Eagle Medium (complete DMEM). The teeth were then washed twice with phosphate buffer saline (PBS) and once with complete DMEM. The teeth were separated into two parts at a dentogingival junction and a sterile endodontic spoon was used to scoop pulp tissue that was then placed onto a glass dish. The tissue was sliced into small pieces with a sterile blade and cultured in complete DMEM at 37 °C and 5% CO₂. Medium was changed every other day. The DPCs grew out of a piece of tissue until 1-2 weeks. Cells were digested and grown in tissue culture flasks. Cells (passage 4) were seeded into a 24-well plate at a density of 2.5×10^4 cells/well. Cells were allowed to adhere for 24 h in complete Dulbecco's Modified Eagle Medium/F12 (DMEM/F12) at 37 °C and 5% CO₂.

MTT assay

Cell viability was determined using the MTT assay. Briefly, the sesame oil or macerated herbal oil was diluted in DMSO to the dilutions of 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128. Then 100 µl of each dilution was added to 10 ml of complete media. Thus, the final concentrations of the oil in 1 ml media were 2.5, 1.25, 0.625, 0.3125, and 0.156 µl

of oil/ml of media. The entire media was discarded and replaced with sesame oil or macerated herbal oil supplemented media. The cell culture was maintained at a temperature of 37 °C in a 5% CO₂ incubator for 48 h. The macerated herbal oil supplemented media was discarded and replaced with 0.5 ml of MTT reagent (0.5 mg in 1 ml media). The reaction was maintained at 37°C and 5% CO₂ for 3 h. The MTT supplemented media was discarded and 1 ml of DMSO was added. The absorbance of the formazan product was measured at 540 nm and the background was corrected at 630 nm. The percentage of the cell viability was compared to that of control, which was cultured in media plus DMSO (100 µl DMSO in 10 ml media). Three replicate assays were performed for the statistical analysis.

TXN gene expression

The expression of TXN gene was previously described in our study.²³ DPCs (passage 4) were seeded into 6-well plates at a density of 5×10^4 cells/well. Sesame oil and macerated herbal oil was diluted in DMEM / F12 at a concentration of 0.2 µl of oil / ml of media (for control group, 0.2 µl of DMSO was diluted in DMEM / F12). Media was added to the seeded cells and then incubated at 37 °C with 5% CO₂ for 21 days. Media was changed twice a week. Cells were trypsinized and washed with PBS; cell pellets were used for further RNA extraction. RNA extraction was performed using High Pure Isolation Kit as suggested by the manufacturer. Total RNA was transcribed into cDNA by the following steps: 16 µl of total RNA was mixed with 4 µl of 5x iScript RT supermix. The reaction took place in an Eppendorf Mastercycler using the following protocol: 1) cDNA priming at 37 °C for 15 min, 2) transcription at 50 °C for 5 min and 3) inactivation at 98 °C for 5 min. NO-RT reaction was prepared as a negative control. DNA elimination was performed using rDNase (Macherey-Nagel). 1 µl of rDNase was added to 10 µl of total RNA following by incubation at 37 °C for 15 min. RT-PCR was performed using SYBR Green Mastermix (SensiFAST SYBR® No-ROX Kit-Bioline). Briefly, cDNA was diluted 1:10 and the 10-µl reaction was composed of 5 µl of SYBR Green RT-PCR master mix, 1 µL of cDNA template, 3.5 µl of distilled water, and 0.25 µM of target-specific primer. RT-PCR was performed in LightCycler480 (Roche) and the polymerase chain reaction protocol consisted of 95 °C pre-incubation for 2 min, followed by 40 cycles of 95 °C for 5 sec, 60 °C for 10 sec, and 72 °C for 10 sec. The melting peak analysis was performed at 95 °C for 5 sec, 65 °C for 1 min, and 97 °C continuous. The cooling step was performed at 40 °C for 30 sec. GAPDH was used as a reference gene. The relative quantification was performed using LightCycler® 480 software 1.5. The list of primers is shown in Table 2.

Table 2 Human target genes & reference gene for Relative Quantitative RT-PCR.

Target genes	Primer sequences (5'→3')
Cyclooxygenase-2 (COX-2)	F: CCCTTGGGTGTCAAAGGTAA
	R: GCCCTCGCTTATGATCTGTC
Interleukin-6 (IL-6)	F: GGTACATCCTCGACGGCATCT
	R: GCCTCTTTGCTGCTTTTAC
Thioredoxin (TXN1)	F: ACGCTGCAGGTGATAAAC
	R: CTGACAGTCATCCACATCTAC
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	F: AAGGGCTCATGACCACAGTC
	R: GGATGACCTTGCCACAG

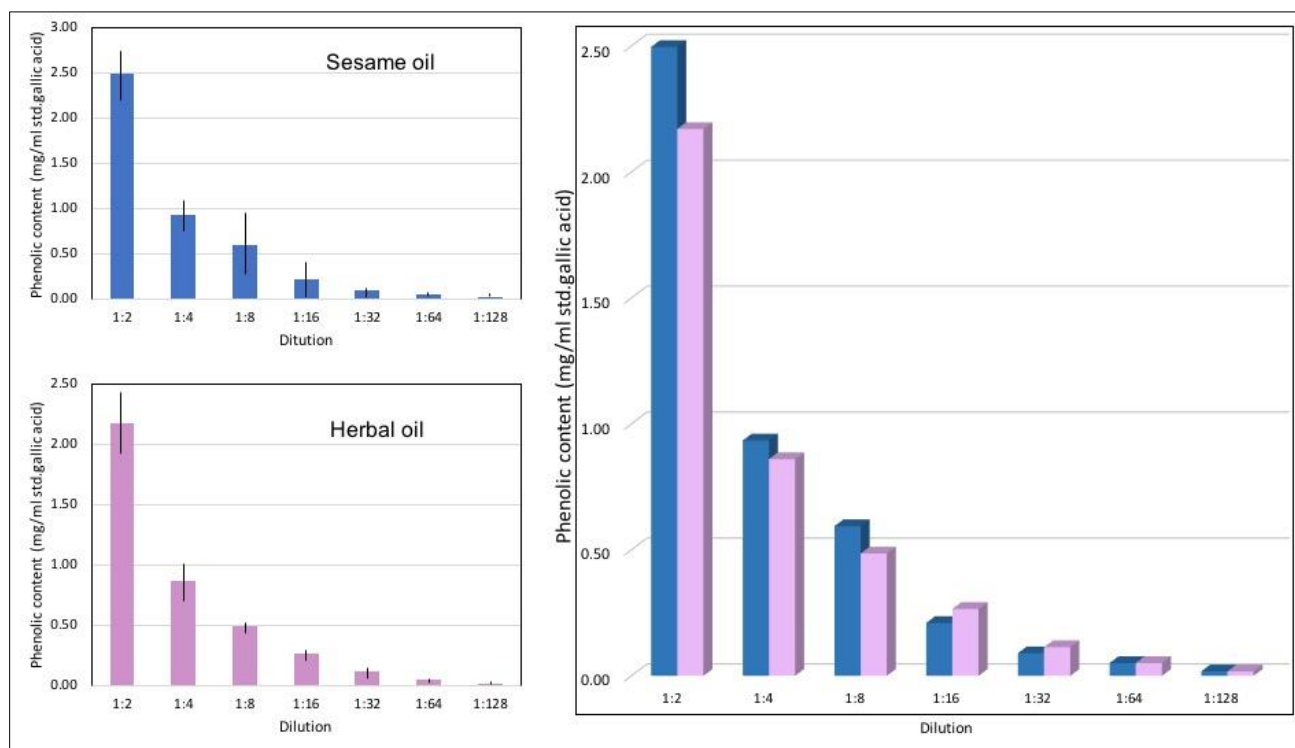
Anti-inflammation assay

DPCs (passage 4) were seeded into 6-well plates at a density of 1×10^5 cells/well and incubated at 37 °C and 5% CO₂ for 48 h. Treatment was performed as follows: cells cultured in DMEM/F12 and vehicle (C), cells cultured in DMEM/F12 with sesame oil 0.2 µl/1 ml of media (S), cells

cultured in DMEM/F12 with macerated herbal oil 0.2 µl/ 1 ml of media (H), cells cultured in LPS 4 µg / ml in DMEM F12 (L), cells cultured in sesame oil 0.2 µl/1 ml and LPS 4 µg/ml in DMEM/F12 (L+S) and cells cultured in macerated herbal oil 0.2 µl/1 ml and LPS 4 µg/ml in DMEM/F12 (L+H). After 48 h, cells were washed and trypsinized. RNA extraction, reverse transcription and real-time PCR were performed as mentioned in the protocol

Results**Total phenolic content**

The phenolic content of sesame oil dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 were equivalent to standard gallic acid concentrations of 2.50, 0.39, 0.59, 0.21, 0.09, 0.05, 0.02 mg / ml, respectively (Figure. 1A). The phenolic content of macerated herbal oil dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 were equivalent to standard gallic acid concentrations of 2.17, 0.86, 0.49, 0.27, 0.11, 0.05, 0.02 mg/ml, respectively (Figure. 1B). The total phenolic content of the sesame and macerated herbal oils did not differ significantly (Figure. 1C).

**Figure 1** Total phenolic content: This figure shows the total phenolic content of sesame oil in comparison with macerated herbal oil using gallic acid as a standard. The phenolic content of the two oils does not differ significantly.

Antioxidant activity**(1) DPPH assay**

From the DPPH assay, the % inhibitions of sesame oil dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 were 27.53, 13.84, 3.88, 2.30, 1.00, 1.00, and 0.62, respectively (Figure. 2A). The % inhibitions of macerated herbal oil dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 were 49.87, 24.13, 10.82, 5.93, 3.97, 2.29, and 2.17, respectively (Figure. 2B). The antioxidant activity of the macerated herbal oil dilutions was significantly higher than the sesame oil (Figure. 2C).

(2) Ferric reducing power activity

This analysis determined the oils' ability to reduce Fe^{3+} to Fe^{2+} . Sesame oil dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32 had reducing activity equivalent to standard BHT concentrations of 0.42, 0.29, 0.12, 0.10, 0.08 $\mu\text{g/ml}$, respectively (Figure. 2D). Macerated herbal oil dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32 had reducing activity equivalent to standard BHT concentrations of 0.47, 0.36, 0.26, 0.19, 0.14 $\mu\text{g/ml}$, respectively (Figure. 2E). The reducing power of macerated herbal oil was statistically higher than sesame oil (Figure. 2F).

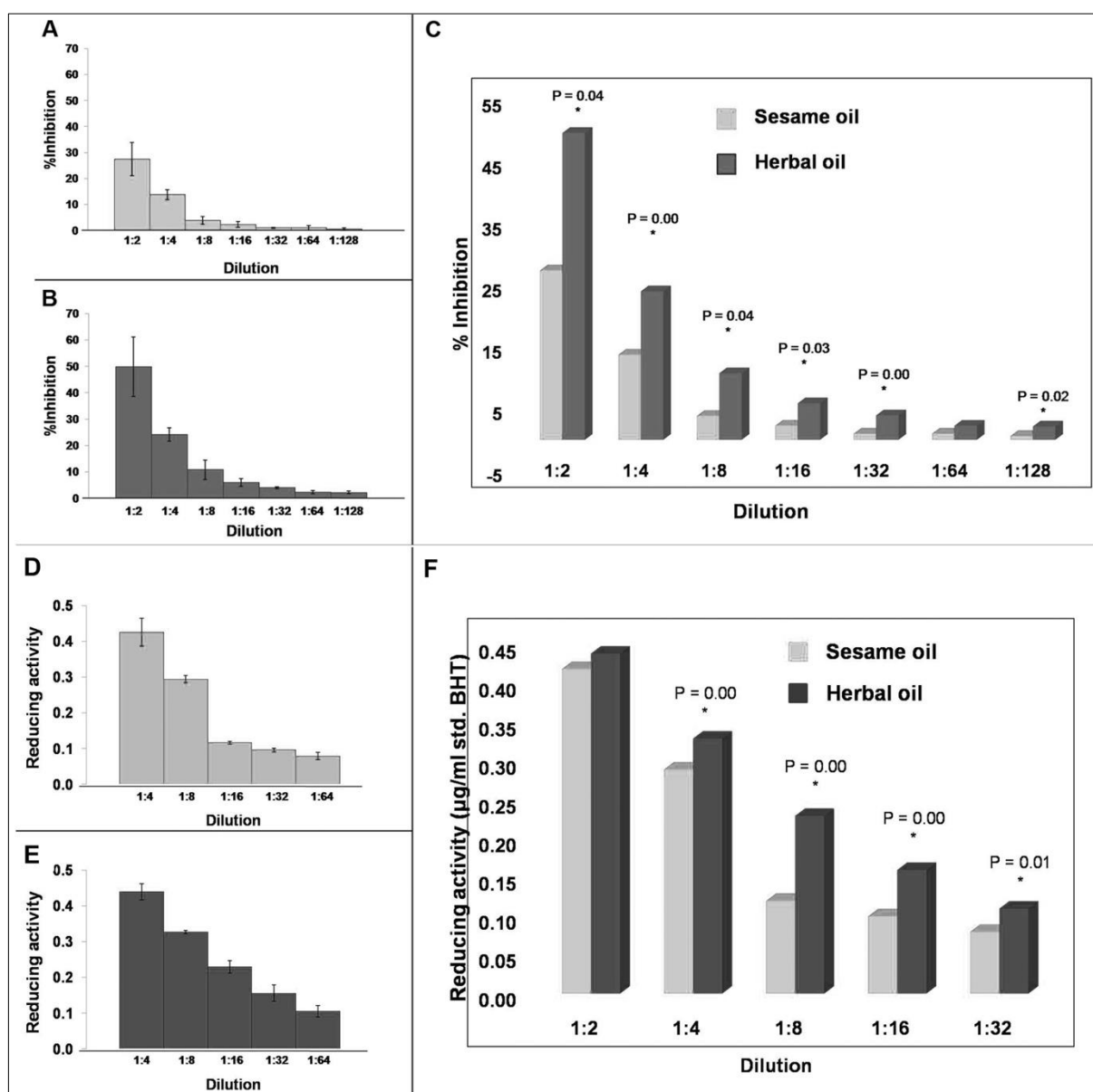


Figure 2 (2A, 2B and 2C) DPPH assay: Macerated herbal oil is significantly more efficient at scavenging DPPH radicals than sesame oil. (2D, 2E and 2F) Ferric reducing power activity: The macerated herbal oil is more capable of reducing Fe^{3+} to Fe^{2+} than sesame oil, with BHT as the standard.

MTT assay

The MTT assay demonstrated that the sesame and macerated herbal oils were not toxic to DPCs; both oils resulted in significant cell proliferation (Figure. 3A & 3B). The % cell viability did not differ significantly between

sesame oil and macerated herbal oil (Figure. 3C) except at the concentration 12.5 μ l of oil/ml of media, sesame oil induced more pronounced cell proliferation than herbal oil.

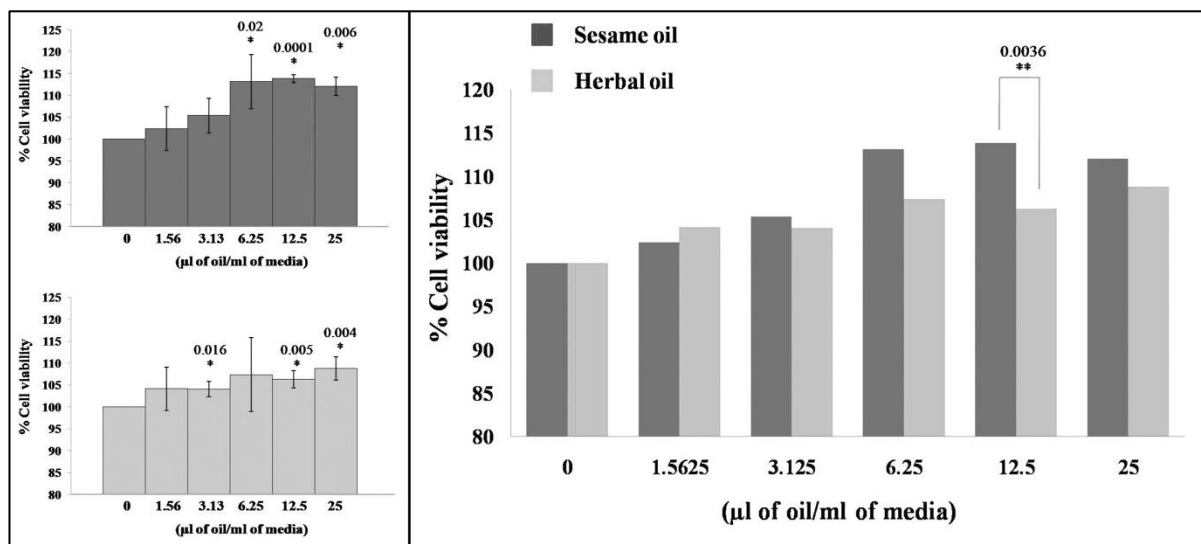


Figure 3 MTT assay: The graphs show the relationship between the concentration of sesame oil & macerated herbal oil versus % viability of DPCs (Figure. 3A & 3B). Sesame oil and macerated herbal oil are not toxic to DPCs and both of them display cell proliferation activity (Figure. 3C).

Antioxidant gene expression

Figure. 4A compared the TXN gene expression of the control group versus DPCs treated with sesame oil and

macerated herbal oil. The expression of TXN gene increased significantly in DPCs cultured in the presence of macerated herbal oils and sesame oil.

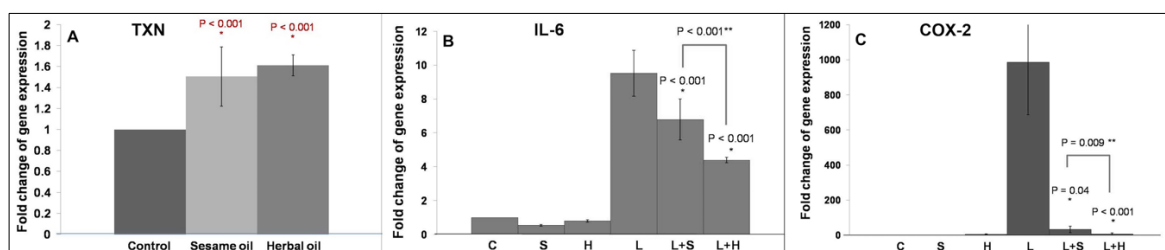


Figure 4 (4A) TXN gene expression: Sesame oil and macerated herbal oil increase TXN gene expression. (4B and 4C) Anti-inflammation assay: Both sesame oil and macerated herbal oil reduce COX-2 and IL-6 gene expression. C = cells cultured in DMEM/F12 with vehicle (DMSO). S = cells cultured in DMEM/F12 with sesame oil (0.2 μ l/ml). H = cells cultured in DMEM/F12 with macerated herbal oil (0.2 μ l/ml). L = cells cultured in DMEM/F12 with LPS (4 μ g/ml). L+S = cells cultured in DMEM/F12 with sesame oil (0.2 μ l/ml) and LPS (4 μ g/ml). L+H = cells cultured in DMEM/F12 with macerated herbal oil (0.2 μ l/ml) and LPS (4 μ g/ml). * = significant at $p < 0.05$.

Anti-inflammatory activity

Sesame oil and macerated herbal oil reduced gene expression of COX-2 and IL-6 in DPCs that had induced inflammation from LPS (4 μ g/ml) compared to the positive control treated with LPS alone (Figure. 4B & 4C). Oil pulling with sesame oil has been shown to significantly reduce plaque-induced gingivitis.²⁵ This study proved that sesame oil inhibited the expression of COX-2 and IL-6, which might be the mechanism of its anti-inflammatory effect. Sesame oil showed anti-inflammatory effects on DPCs, without cytotoxicity. Moreover, COX-2 and IL-6 decreased significantly, when macerated herbal oil was supplemented to LPS-treated cells.

Discussion

Herbal medicine can be applied to improve the efficacy of sesame oil. Adding herbs to hot sesame oil by a method called hot oil maceration produces macerated herbal oil. This study found that sesame oil contains phenolic compounds, and adding herbs does not significantly affect the total phenolic content. However, a previous report indicated that the efficiency of maceration for extracting phenolic compounds is low.²⁶ Sesame and macerated herbal oils exhibited antioxidant activity through scavenging of DPPH radicals; they also showed ferric-reducing properties. The macerated herbal oil's ability to scavenge DPPH radicals and its reducing properties are statistically higher than the sesame oil.

The total phenolic contents of sesame oil and macerated herbal oil were not different. However, antioxidant and anti-inflammatory activities of macerated herbal oil were superior which indicated that the potential antioxidant and anti-inflammatory activities in macerated herbal oil might come from the combination of phenolic compounds in sesame oil and other non-phenolic compounds from the added herbs.

It appears that sesame oil induced dental pulp cell proliferation. These results support that sesame oil has potential for oil pulling; adding herbs may provide even more benefits. Our previous study showed that macerated herbal oil reduced inflammation in HMSCs.²³ In the present, study the proportion of each component was modified. The previous study was found that macerated herbal oil has toxicity at the concentration ≥ 2.5 $\mu\text{l/ml}$ in complete DMEM.²³ Thus, menthol, camphor, and borneol were excluded from this study and the toxicity was not present anymore (Figure. 3). The macerated herbal oil slightly induced cell proliferation. Moreover, this study demonstrated that treatment with sesame or macerated herbal oils significantly induced the antioxidant gene (Figure. 4A). This result correlated well with previous studies by Yeh et al. that demonstrated that phenolic acid induced antioxidant enzyme expression.^{27, 28}

This study demonstrated that macerated herbal oil is a better antioxidant and anti-inflammatory than sesame oil. The anti-microorganism assay was performed with *Streptococcus mutans* and *Candida albican* (data not shown); neither sesame oil nor macerated herbal oil demonstrated anti-microorganism activity (data not shown). The sesame oil and macerated herbal oil did not inhibit *Streptococcus mutans* and *Candida albican* (data not shown). This result is supported by Asokan et al., who found that sesamin and sesamolin isolated from sesame oil showed no antibacterial effect against oral microorganisms, such as *Streptococcus mutans*, *Streptococcus mitis* and *Streptococcus viridians*.²⁹ This result also correlated with Jauhari et al., who found that oil pulling does not provide any additional benefit as an antimicrobial agent in reducing the bacterial colonization of an individual.³⁰ However, it is possible that the saponification and emulsification process, which enhances its mechanical cleaning action, should be considered as an underlying mechanism.²⁹ Given this mechanism, together with the anti-inflammatory effect, sesame oil can be used in oil pulling, with the addition of herbs increasing its efficacy.

In conclusion, the macerated herbal oil showed no statistical difference in total phenolic content compared to sesame oil. The macerated herbal oil did, however, display superior antioxidant activity compared to sesame oil. Neither sesame oil nor macerated herbal oils were cytotoxic to DPCs, and both induced cell proliferation and increased TXN gene expression in DPCs. The presence of macerated herbal oil and sesame oil attenuated the LPS-induced expression of COX-2 and IL-6 genes. Macerated herbal oil reduced expression of COX-2 and IL-6 better than sesame oil. Adding herbs to produce macerated herbal oil improved the efficiency of sesame oil, offering potential as an alternative oral health care product.

Conflict of interest:

The authors have no conflicting interests.

Acknowledgements

This work was supported by the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand.

References

- [1] Asokan S. Oil pulling therapy. Indian J Dent Res. 2008;19(2): 169. doi: 10.4103/0970-9290.40476.
- [2] Asokan S, Rathan J, Muthu MS, Rathna PV, Emmadi P, Raghuraman, et al. Effect of oil pulling on Streptococcus mutans count in plaque and saliva using Dentocult SM Strip mutans test: a randomized, controlled, triple-blind study. J Indian Soc Pedod Prev Dent. 2008;26(1): 12-7. doi: 10.4103/0970-4388.40315.
- [3] Peedikayil FC, Sreenivasan P, Narayanan A. Effect of coconut oil in plaque related gingivitis - A preliminary report. Niger Med J. 2015;56(2): 143-7. doi: 10.4103/0300-1652.153406.
- [4] Asokan S, Kumar RS, Emmadi P, Raghuraman R, Sivakumar N. Effect of oil pulling on halitosis and microorganisms causing halitosis: a randomized controlled pilot trial. J Indian Soc Pedod Prev Dent. 2011;29(2): 90-4. doi: 10.4103/0970-4388.84678.
- [5] Sood P, Devi M A, Narang R, V S, Makkar DK. Comparative efficacy of oil pulling and chlorhexidine on oral malodor: a randomized controlled trial. J Clin Diagn Res. 2014;8(11): ZC18-21. doi: 10.7860/JCDR/2014/9393.5112.
- [6] Makinde FM, Akinoso R. Comparison between the nutritional quality of flour obtained from raw, roasted and fermented sesame (Sesamum indicum L.) seed grown in Nigeria. Acta Sci Pol Technol Aliment. 2014;13(3): 309-19.
- [7] Fostok SF, Ezzeddine RA, Homaidan FR, Al-Saghir JA, Salloum RG, Saliba NA, et al. Interleukin-6 and Cyclooxygenase-2 downregulation by fatty-acid fractions of Ranunculus constantinopolitanus. BMC Complement Altern Med. 2009;9: 44. doi: 10.1186/1472-6882-9-44.
- [8] Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, et al. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J Clin Invest. 1998;101(2): 311-20.
- [9] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta. 2011;1813(5): 878-88. doi: 10.1016/j.bbam-cr.2011.01.034.
- [10] Sastravaha G, Yotnuengnit P, Booncong P, Sangtherapitkul P. Adjunctive periodontal treatment with Centella asiatica and Punica granatum extracts. A preliminary study. J Int Acad Periodontol. 2003;5(4): 106-15.

- [11] Kadam A, Prasad BS, Bagadia D, Hiremath VR. Effect of Ayurvedic herbs on control of plaque and gingivitis: A randomized controlled trial. *Ayu*. 2011;32(4): 532-5. doi: 10.4103/0974-8520.96128.
- [12] Kumar G, Jalaluddin M, Rout P, Mohanty R, Dileep CL. Emerging trends of herbal care in dentistry. *J Clin Diagn Res*. 2013;7(8): 1827-9. doi: 10.7860/JCDR/2013/6339.3282.
- [13] Bua-in S, Paisooksantivatana Y. Essential Oil and Antioxidant Activity of Cassumunar Ginger (Zingiberaceae: *Zingiber montanum* (Koenig) Link ex Dietr.) Collected from Various Parts of Thailand. *Kasetsart J (Nat Sci)*. 2009;43: 467 - 75.
- [14] Zakaria ZA, Mohamad AS, Ahmad MS, Mokhtar AF, Israf DA, Lajis NH, et al. Preliminary analysis of the anti-inflammatory activity of essential oils of *Zingiber zerumbet*. *Biol Res Nurs*. 2011;13(4): 425-32. doi: 10.1177/1099800410386590.
- [15] Stenholm A, Göransson U, Bohlin L. Bioassay-guided supercritical fluid extraction of cyclooxygenase-2 inhibiting substances in *Plantago major* L. *Phytochem Anal*. 2013;24(2): 176-83. doi: 10.1002/pca.2398.
- [16] Abirami A, Gunasekaran Siddhuraju, Perumal. In vitro antioxidant, anti-diabetic, cholinesterase and tyrosinase inhibitory potential of fresh juice from *Citrus hystrix* and *C. maxima* fruits. *Food Science and Human Wellness*. 2014;3(1): 16-25. doi: 10.1016/j.fshw.2014.02.001.
- [17] Singtothong C, Gagnon MJ, Legault J. Chemical composition and biological activity of the essential oil of *Amomum biflorum*. *Nat Prod Commun*. 2013;8(2): 265-7.
- [18] Verma RS, Joshi N, Padalia RC, Singh VR, Goswami P, Verma SK, et al. Chemical composition and antibacterial, antifungal, allelopathic and acetylcholinesterase inhibitory activities of cassumunar-ginger. *Journal of the Science of Food and Agriculture*. 2018;98(1): 321-7. doi: 10.1002/jsfa.8474.
- [19] Lee CY, Chen SP, Su CH, Ho YC, Yang ML, Lee SS, et al. Zerumbone from *Zingiber zerumbet* Ameliorates Lipopolysaccharide-Induced ICAM-1 and Cytokines Expression via p38 MAPK/JNK-IkB/NF-κB Pathway in Mouse Model of Acute Lung Injury. *Chin J Physiol*. 2018;61(3): 171-80. doi: 10.4077/CJP.2018.BAG562.
- [20] Entezari Heravi N, Hosseini S, Naji Ebrahimi Yazd Z, Shafei MN, Ebrahimzadeh Bideskan A, Shahraki S, et al. Doxorubicin-induced renal inflammation in rats: Protective role of *Avicenna* J Phytomed. 2018;8(2): 179-87.
- [21] Sun S, Phrutivorapongkul A, Dibwe DF, Balachandran C, Awale S. Chemical Constituents of Thai *Citrus hystrix* and Their Antiausterity Activity against the PANC-1 Human Pancreatic Cancer Cell Line. *J Nat Prod*. 2018. doi: 10.1021/acs.jnatprod.8b00405.
- [22] Ayadi MA, Grati-Kamoun N, Attia H. Physico-chemical change and heat stability of extra virgin olive oils flavoured by selected Tunisian aromatic plants. *Food Chem Toxicol*. 2009;47(10): 2613-9. doi: 10.1016/j.fct.2009.07.024.
- [23] Kantawong F, Singhatong S, Srilamay A, Boonyuen K, Mooti N, Wanachantararak P, et al. Properties of macerated herbal oil. *Bioimpacts*. 2017;7(1): 13-23. doi: 10.15171/bi.2017.03.
- [24] Kantawong F, Thaweenan P, Mungkala S, Tamang S, Manaphan R, Wanachantararak P, et al. Mucus of *Achatina fulica* stimulates mineralization and inflammatory response in dental pulp cells. *Turk J Biol*. 2016;40(2): 353-9. doi:10.3906/biy-1505-29.
- [25] Asokan S, Emmadi P, Chamundeswari R. Effect of oil pulling on plaque induced gingivitis: a randomized, controlled, triple-blind study. *Indian J Dent Res*. 2009;20(1): 47-51.
- [26] Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. *Molecules*. 2013;18(2): 2328-75. doi: 10.3390/molecules18022328.
- [27] Yeh CT, Yen GC. Induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance-associated protein 3 mRNA expression. *J Nutr*. 2006;136(1): 11-5.
- [28] Yeh CT, Ching LC, Yen GC. Inducing gene expression of cardiac antioxidant enzymes by dietary phenolic acids in rats. *J Nutr Biochem*. 2009;20(3): 163-71. doi: 10.1016/j.jnutbio.2008.01.005.
- [29] Asokan S, Rathinasamy TK, Inbamani N, Menon T, Kumar SS, Emmadi P, et al. Mechanism of oil-pulling therapy - in vitro study. *Indian J Dent Res*. 2011;22(1): 34-7. doi: 10.4103/0970-9290.79971.
- [30] Jauhari D, Srivastava N, Rana V, Chandna P. Comparative Evaluation of the Effects of Fluoride Mouthrinse, Herbal Mouthrinse and Oil Pulling on the Caries Activity and Streptococcus mutans Count using Oratest and Dentocult SM Strip Mutans Kit. *Int J Clin Pediatr Dent*. 2015;8(2): 114-8. doi: 10.5005/jp-journals-10005-1295.