Infection - pain in oral cavity and alternative managements

PhD thesis

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Introduction

Pain is an unpleasant sensory and emotional experience associated with actual and potential tissue damages. Tissue injuries and/or infection cause pain through inflammatory process via inflammatory mediators. Activated immune cells secrete cytokines, histamine, bradykinin, prostaglandin and leukotriene which decrease action potential threshold of nociceptive neurons. As a result, afferent nociceptive neurons are readily generating pain signals and transmitting to higher centers. Furthermore, microbial pathogenic ligands can bind to pathogen recognition receptors expressed on nociceptors, resulting in activation and sensitization of nociceptive neurons.

The oral cavity harbors more than 700 species of non-pathogenic and pathogenic microbes. In health, each microbe is in a commensal stage between microbes and host. However, when an imbalanced interplay between microbe-microbe or microbe-immune occurs, it can lead to diseases. Some bacteria, virus and fungi that cause diseases in the orofacial areas have been to found to stimulate and activate nociceptors directly (Table 1).

Table 1. Pathogens caused diseases in the orofacial area can sensitize nociceptors.

Pathogen name	Disease	Infection site	Neuronal sensitization mechanism
Bacteria			
Porphyromonas gingivalis	Periodontal disease	Oral cavity	LPS sensitizes TRPV1
Virus			
Herpes simplex virus	Herpes labialis/ gingivostomatitis	Trigeminal ganglia	Nociceptor sensitization
Varicella zoster virus	Orofacial herpes zoster	Trigeminal ganglia	PHN
Fungus			
Candida albicans	Candidiasis	Skin/ oral cavity	Zymosan sensitizes nociceptive neurons

LPS: lipopolysaccharide, PHN: postherpetic neuralgia, TRPV1: transient receptor potential channel, vanilloid subtype 1. Adapted from Chiu IM. (2018) Infection, Pain, and Itch. Neurosci. Bull., 34: 109-119.

Periodontal diseases

Periodontal diseases encompass a wide variety of inflammation of gingiva, bone and tooth supporting structures, initiated by bacterial dysbiosis.

Bacterial associated with periodontal diseases are dominantly gram-negative bacteria, such as *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Prevotella intermedia,* and *Fusobacterium nucleatum*. In addition, *Aggregatibacter actinomycetemcomitans* is considered as a key pathogen of early-onset periodontitis. Typically, periodontal diseases can cause mild to moderate, episodic or persistent dull pain due to infection and inflammation.

Probiotics has been introduced for patients with periodontal diseases in order to maintain and prolong well balanced oral microflora. Many studies have reported the beneficial effect of probiotics against dental caries, halitosis, and periodontal disease. However, the benefits of probiotics in periodontal diseases are questionable. Some trials showed a significant decrease of periodontal pathogens and improved periodontal clinical parameters after using probiotics, but not in some trails. In addition, the evidence from those mentioned studies is very weak, since the studied sample sizes were relatively small and limitations inherited in each study. Therefore, we aimed to study the effect of probiotics on periodontal pathogenic bacteria on the data from available randomized clinical trials.

Orofacial herpes zoster

Orofacial herpes zoster is an infection at face and oral cavity cause by Varicella zoster virus. The infection causes damages to the infected neurons, nerve fibers, and target tissues which innervated by those infected nerves. Consequently, structures and functions of both peripheral and central nervous system have been altered, especially sensory function. Pain, hyperalgesia, and allodynia are the most common complaint in herpes zoster infection, since the virus causes peripheral and central sensitization, and abnormal reorganization of nociception.

The herpes zoster-associated pain has been categorized into acute zoster pain (acute herpetic neuralgia), subacute zoster pain (subacute herpetic neuralgia), and postherpetic neuralgia (PHN) since the onset of the rash. PHN is complicated and intractable pain that usually develops after infection, especially in elders.

Gabapentinoids (Gabapentin and Pregabalin) are derivative of GABA which bind to the α2-δ sub-unit of voltage-dependent calcium channels. They have ability to inhibit ectopic discharges from peripheral

nerve injuries, to suppress neuralgia and sensitization, and to modulate of GABAergic, glutaminergic and monoaminergic function. These substances have been used for preventing the development of PHN from the aspect of effectively controlling pain and neuronal sensitization. However, the available evidence showed a contradictory outcome. Therefore, we aimed to study the effectiveness of gabapentinoids in the reduction of acute herpes zoster pain occurrence after herpes zoster infection.

Objectives

1. To investigate the effect of probiotics in reducing periodontal pathogenic bacteria.
2. To investigate the effect of gabapentinoids in preventing the occurrence of acute herpes zoster pain after herpes zoster infection.

In order to draw a conclusion from different available evidence, all objectives were investigated using systematic review and meta-analysis.

Methods

Protocol and registration

Both meta-analyses were reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA), and were registered in the international prospective register of systematic review (PROSPERO).

Eligibility criteria

A PICO (patient, intervention, control, and outcomes) formats were constructed following the clinical question whether the orally administered probiotics decreases the level of pathogenic periodontal bacteria in saliva and dental plaque.

Orally administered probiotics decreases the level of pathogenic periodontal bacteria in saliva and dental plaque	
P	Patients with periodontal diseases
I	Orally administered probiotics
C	Placebo or no treatment
O	Amount of pathogenic periodontal bacteria in saliva, supra- and sub-gingival plaque

Inclusion and exclusion criteria

The inclusion criteria were randomized controlled trials that used orally administered probiotics versus placebo or no treatment in patients with periodontal disease. The studies used antibiotics were excluded.

Searching strategy and information sources

A systematic search was done from PubMed, Cochrane Central Register of Controlled Trials (CENTRAL), EMBASE, and Web of Science, from inception to 7 June 2020. The language of literatures was limited to English. The keyword used for the search was (probiotic **AND** (periodontal disease **OR** periodontitis **OR** gingivitis **OR** plaque **OR** saliva)).

Data extraction

Data were extracted by two independent reviewers on first author, year of publication, number and characteristics of patients, pretreatment, probiotic strain, dose, form, instruction and duration, comparator, and number of periodontal pathogens such as *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *F. nucleatum*, and *T. forsythia* in saliva, and supra- and sub-gingival plaque.

Assessment of risk of bias

The Cochrane Risk of Bias Tool was assessed in each included study in order to eliminate the bias in the findings and transparent the final results. All included studies was assessed according to various domains. Each study was categorized as a low risk of bias when all domain were low risk; an unclear risk of bias when there was at least one unclear risk of bias; and a high risk of bias when there was at least one high risk of bias.

Assessment of heterogeneity

The certainty and strength of evidence for each outcome was evaluated according to the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach using GRADEPro® (McMaster University, Hamilton, Canada). It gave the certainty of evidence into high, moderate, low, or very low.

Statistical analysis

The amount of bacteria before and after the treatment was calculated for the standardized mean difference (SMD). The SMD values from each study were pooled using the random effects model with the DerSimonian-Laird estimator and graphically displayed on forest plots. The summary SMD estimation, and 95% CI were calculated. $P < 0.05$ was considered as a significant difference from summary SMD = 0. The statistical heterogeneity was analyzed using the I^2 statistic and χ^2 statistic to ascertain probability values; $p < 0.1$ was defined as indicating significant heterogeneity.

Results

The result of literature search and the selection of study

The comprehensive literature search from databases yielded 2,210 records. No additional record was found through the bibliographic references of review articles. After removing duplicates, there were 1,281 records remaining and they were screened for titles and abstracts. Twenty-five remaining records were assessed for eligibility. Nine articles were excluded due to non-randomized controlled trials, non-periodontal disease participants, sub-gingivally administration, and non-living bacteria. Other two studies were also excluded because they did not fit the aim of this meta-analysis from measuring total bacterial numbers and obligate anaerobes. Finally, fourteen articles were included in qualitative analysis, and out of these, nine were suitable for quantitative analysis.

From fourteen included for qualitative analysis, five studies were excluded because of no periodontal pathogen number obtained after contacting the authors, unspecified participants with periodontal disease, used combinations of probiotics and antibiotics, and delay of probiotics administration. The remaining nine articles were included for quantitative analysis.

Characteristics of included studies

All of the included studies are RCTs, but they were different in details. Four studies were open, controlled, parallel RCTs; eight investigations were double-blinded RCTs; one study was double-blinded, crossover RCT, and the last one was double-blinded, split-mouth RCT. Each study inherited its uniqueness experimental design, such as different probiotic strain, forms of probiotics, instruction of use, duration of study, and measurement of different periodontal pathogenic bacteria. For example, six studies used molecular PCR method to measure the amount of periodontal pathogenic bacteria, while the rest used conventional cultivation method. Seven studies provide a scaling and root planning to participants before using probiotics, but the other two did not provide any professional cleaning.

The risk of bias assessment

All studies detailed the method of randomization and they were rated as a low risk of bias, except, one study did not specified the clearly method. Thus, it was rated as questionable risk of bias. Five studies were determined as high risk of bias in allocation concealment domain because of no blinding of staffs. The performance bias domain was determined as high risk in four studies due to lack of blinding participants and involved personnel, and questionable risk in one study because of a chance of unblind personnel. The attrition bias domain which assesses the incomplete outcome data was rated as high risk for four studies due to an incomplete report without further explanation. Selective reporting bias from three studies were rate as high risk of bias because they did not report all prespecified outcomes. The results of risk of bias assessment are summarized and detailed in Figure 1.

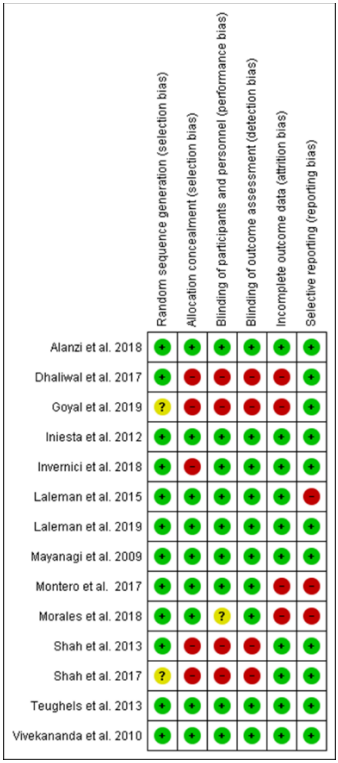

Figure 1. A summary of the risk of bias of included studies.

Review authors' judgements about each methodological quality presented in different aspect from each included study. From Sang-Ngoen T et al. (2021) Orally Administered Probiotics Decrease Aggregatibacter actinomycetemcomitans but Not Other Periodontal Pathogenic Bacteria Counts in the Oral Cavity: A Systematic Review and Meta-Analysis. Front Pharmacol, 12: 682656: 1-14.

Certainty of evidence assessment

The level of certainty of evidence was done using the GRADE approach on the efficacy of orally-administered probiotics to reduce the quantity of harmful periodontal bacteria. The outcomes from different pathogenic periodontal bacteria and duration of measurement revealed very low grade in all assessments due to the presence of serious a risk of bias, inconsistency and imprecision.

The result of the meta-analysis

A. actinomycetemcomitans, *P. gingivalis*, *P. intermedia*, *F. nucleatum*, and *T. forsythia* were measured after probiotics treatment in different designated follow-up duration (four-, eight-, or twelve-week). Only the *A. actinomycetemcomitans* showed the significantly positive effect of number reduction after four week of probiotics treatment, disregarding locations of samples collected (SMD= -0.28; 95%CI= -0.56 to -0.01; p=0.045; heterogeneity: I^2=36.5%, p=0.150). However, the saliva, sub- or supra-gingival plaque of *A. actinomycetemcomitans* at four week did not show a significant difference between probiotics treated group and control group (Figure 2). In addition, the probiotics treated group was not significant difference from the control group in overall result, saliva, sub-or supra-gingival plaque at eight week (Figure 3). With disregarding or regarding locations of samples collected, the number of *P. gingivalis*, *P. intermedia*, *F. nucleatum*, and *T. forsythia* after treatment were not significant different between probiotics treatment and control treatment groups at four-, and eight-week. Further forest plots representing the SMD of *P. gingivalis*, *P. intermedia*, *F. nucleatum*, and *T. forsythia* after probiotics treatment can be found in the publication related to this thesis.

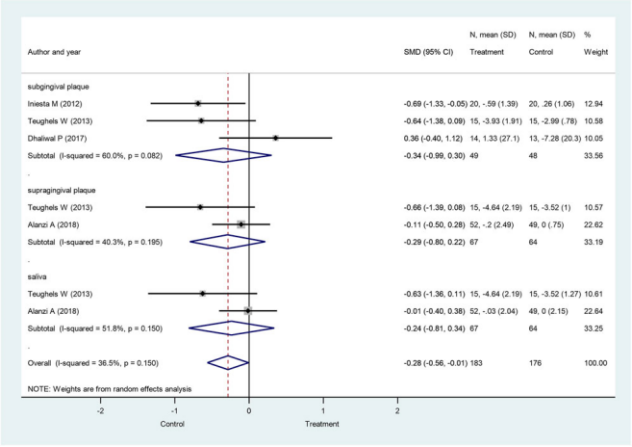

Figure 2. Forest plot analysis of the change in *A. actinomycetemcomitans* at 4 weeks.

The overall result of the standardized mean difference indicated a significant decrease of *A. actinomycetemcomitans* in the probiotic treatment over the control. From Sang-Ngoen T *et al.* (2021) Orally Administered Probiotics Decrease Aggregatibacter actinomycetemcomitans but Not Other Periodontal Pathogenic Bacteria Counts in the Oral Cavity: A Systematic Review and Meta-Analysis. Front Pharmacol, 12: 682656: 1-14.

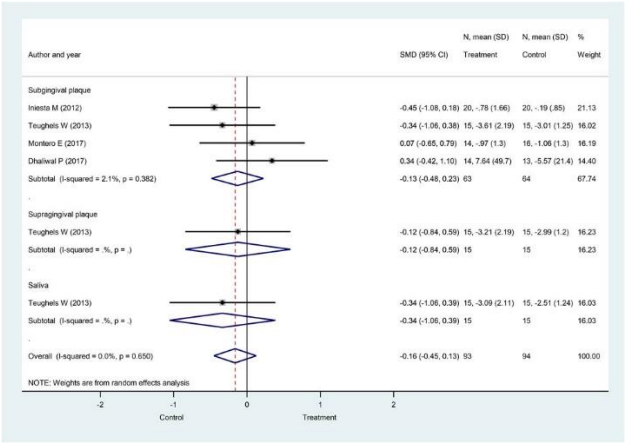

Figure 3 Forest plot analysis of the change in *A. actinomycetemcomitans* at 8 weeks.

The overall result of the standardized mean difference indicated a significant decrease of *A. actinomycetemcomitans* in the probiotic treatment over the control. From Sang-Ngoen T *et al.* (2021) Orally Administered Probiotics Decrease Aggregatibacter actinomycetemcomitans but Not Other Periodontal Pathogenic Bacteria Counts in the Oral Cavity: A Systematic Review and Meta-Analysis. Front Pharmacol, 12: 682656: 1-14.

Conclusion

The results from our meta-analysis suggested that orally administered probiotics have an ability to reduce numbers of pathogenic periodontal bacteria – *A. actinomycetemcomitans* at 4 week, but not at 8 week, after the initiation of treatment in a pooled analysis. The amount of *A. actinomycetemcomitans* count in subgroup analyses from sub-gingiva, supra-gingiva and saliva tends to decrease after probiotics treatment. However, probiotics have no beneficial effect in reducing *P. gingivalis, P. intermedia*, *F. nucleatum*, and *T. forsythia*. The use of orally administered probiotics as an adjunct to the conventional scaling and root planning could reduce a specific strain of periodontal pathogenic bacteria in healthy periodontal patients. However, due to distinct heterogeneity among the available RCTs, standardized clinical protocol is needed to further evaluate the effect of various probiotics on periodontal pathogens.

Methods

Protocol and registration

Both meta-analyses were reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA), and were registered in the international prospective register of systematic review (PROSPERO).

Eligibility criteria

A PICO (patient, intervention, control, and outcomes) formats were constructed following the clinical question whether gabapentinoids reduce the occurrence of the herpes zoster pain.

Gabapentinoids reduce the occurrence of the herpes zoster pain	
P	Patients with herpes zoster infection
I	Gabapentinoids
C	Placebo or no treatment
O	The presence of acute herpes zoster pain

Inclusion and exclusion criteria

The inclusion criteria were randomized controlled trials that used orally administered gabapentinoids versus placebo or no treatment in patients with herpes zoster infection. The other routes of administration were excluded from this study.

Searching strategy and information sources

A systematic search was conducted in PubMed, Web of Science, Ovid, Scopus and EMBASE databases from inception to 8 August 2018. The language of literatures was limited to English. The keyword used for the search was (herpes zoster **AND** ("gamma-aminobutyric acid" **OR** "gaba" **OR** "gabapentin" **OR** neurontin **OR** "pregabalin")).

Data extraction

Data were extracted by two independent reviewers on first author, year of publication, country, number of centers, design of study, size of population, intervention and duration of intervention, demographic data, distribution and severity of lesion, number of dropout patients, reasons for dropouts, the presence of herpes zoster-associated pain after treatments,

adverse events, and other relevant information that is not specific in the study protocol.

Assessment of risk of bias

The Cochrane Risk of Bias Tool was assessed in each included study in order to eliminate the bias in the findings and transparent the final results. All included studies was assessed according to various domains. Each study was categorized as a low risk of bias when all domain were low risk; an unclear risk of bias when there was at least one unclear risk of bias; and a high risk of bias when there was at least one high risk of bias.

Assessment of heterogeneity

The certainty and strength of evidence for each outcome was evaluated according to the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach using GRADEPro® (McMaster University, Hamilton, Canada). It gave the certainty of evidence into high, moderate, low, or very low.

Statistical analysis

The number of patients with observed presence of pain in the test and the control group was used to calculate the odds ratio (OR). The ORs were pooled using the random-effect model with the DerSimonian-Laird estimator and graphically displayed on forest plots. Summary OR estimation, and 95% confidence interval (CI) were calculated. $p < 0.05$ was considered as a significant difference from summary OR=1. The statistical heterogeneity was analyzed using the I^2 statistic and χ^2 statistic to ascertain probability values; $p<0.05$ indicates potential risks of bias.

Results

The result of literature search and the selection of study

The comprehensive literature search from databases yielded 4,888 records. No additional record was found through the bibliographic references of review articles. After removing duplicates, there were 3,130 records remaining and they were screened for titles and abstracts. Six records were relevant to the meta-analysis. Out of these, three records were excluded because one was the uncontrolled trial; another one was a retrospective study; while the third one was an ongoing trial. The remaining three suitable records were extracted for data.

Characteristics of included studies

All included studies were single center RCTs. Each of studies exhibited some differences among each other such as the age of participants, the dosage and duration of treatment, and the use of antiviral agent. Only one study reported additional outcome on the quality of life. one study included participants older than 50 years old, while another study included participants age from 30 – 80 years old. Furthermore, participants from one studies received antiviral agents with the study drug within 72 hour after the onset of zoster rash, but participants in another study received only the study drug in a delay period (7-14 days after the onset of rash).

The risk of bias assessment

Although all studies stated that they were randomized trials, none of them has detailed in the method of randomization. Therefore, the selection bias in both random sequence generation and allocation concealment were rated as questionable risk of bias. All studies provided insufficiently information used for blinding of participants and personnel, thus, they were determined as questionable risk of bias in the domain of performance bias. A description of blinding outcome assessment method was described only in one study, therefore, it exhibited low risk of bias in the domain of detection bias. Two studies were judged to have a high rate of incomplete outcome data (attrition bias domain) because of high drop out rate and no dropout reason. A low risk of reporting bias was determined in two studies, while another one was rated as high risk because all pre-specified outcomes were not reported. To sum up, all studies exhibited high risk of bias because there was at least

one high risk of bias in the key domains. The results of risk of bias assessment are summarized and detailed in Figure 4.

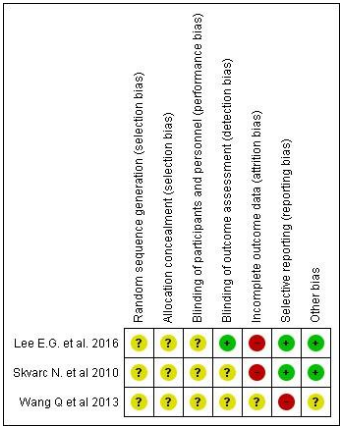

Figure 4. A summary of the risk of bias of included studies.
Review authors' judgements about each methodological quality presented in different aspect from each included study. From **Sadaeng W** et al. (2020) gamma-Aminobutyric Acid and Derivatives Reduce the Incidence of Acute Pain after Herpes Zoster - A Systematic Review and Meta-analysis. Curr Pharm Des, 26:3026-38.

Certainty of evidence assessment

The level of certainty of evidence was done using the GRADE approach on the efficacy of gabapentinoids to reduce the acute herpes zoster pain occurrence. The result reveled very low grade because of the presence of very high risk of bias and publication bias.

The result of meta-analysis

The presence of acute zoster pain after treatment

The forest plot showed the pooled odds ratios of events occurred after gabapentinoids treatment (Figure 5). The result indicated that the occurrence of acute herpes zoster pain in the gabapentinoids group was significantly lower compared to the placebo group (OR=0.36; 95% CI= 0.14 to 0.93; p=0.035; heterogeneity: I^2=40.7%, p=0.186). This suggested that

gabapentinoids could prevent acute zoster pain in patients after herpes zoster infection.

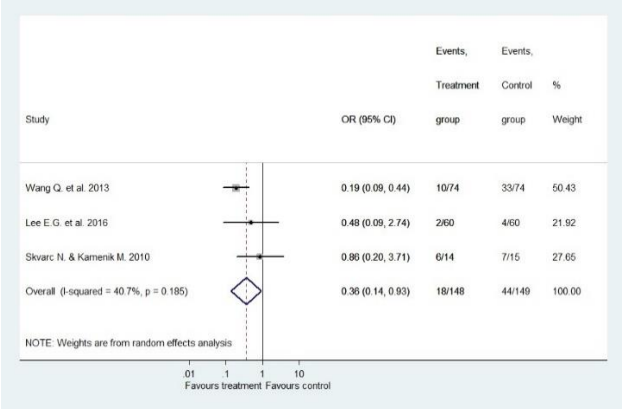

Figure 5. Forest plot analysis of the presence of acute zoster pain.
Overall results of odds ratio indicated a preventive effect from acute zoster pain in the gabapentinoids group over the placebo group. From **Sadaeng W** et al. (2020) gamma-Aminobutyric Acid and Derivatives Reduce the Incidence of Acute Pain after Herpes Zoster - A Systematic Review and Meta-analysis. Curr Pharm Des, 26:3026-38.

Presences of adverse events during treatment

The adverse events were noted in only two studies. They reported different aspects of adverse events, but they shared some common aspect such as fatigue, constipation, and dizziness. None of them were different between gabapentinoids group and control group. The meta-analysis on this basis was not performed due to insufficient data.

The quality of life

The dermatologic life quality index was assessed and reported in only one study. The patients suffering from HZI were asked to determine how skin problem affects life. The quality of life between the gabapentinoids treated group and the control group was not significantly difference. The meta-analysis on this basis was not performed due to insufficient data

Conclusion

The results from our meta-analysis indicated that administration of gabapentinoids reduce the occurrence of acute herpes zoster pain after the healing of rash. Gabapentinoids also help improving the quality of life in patients who have suffered from pain during and after herpes zoster infection. However, patients could experience some adverse events, such as dizziness, which may be reasons for refusing treatment. This study provides preliminary evidence in the prevention of the development of PHN from the anti-sensitization aspect as the use of gabapentinoids can control pain which leads to the prevention of pain sensation. However, currently available evidence on this matter is weak. Therefore, additional, well designed randomized clinical trials are needed, as well as a long term study with a higher dosage of gabapentinoids.

Final conclusions

1. Orally administered probiotics as an adjunct to standard periodontal treatment have an ability to reduce the number of pathogenic periodontal bacterial – *A. actinomycetemcomitans* at 4 week after the initiation of treatment.

2. The administration of gabapentinoids reduce the occurrence of acute herpes zoster pain after the healing of rash.

List of own publications related to the PhD thesis

1. **Sadaeng W**, Marta K, Matrai P, Hegyi P, Toth B, Nemeth B, Czumbel LM, Sang-Ngoen T, Gyongyi Z, Varga G, Revesz P, Szanyi I, Karadi K, Gerber G. (2020). gamma-Aminobutyric Acid and Derivatives Reduce the Incidence of Acute Pain after Herpes Zoster - A Systematic Review and Meta-analysis. Curr Pharm Des, 26:3026-38. **IF: 3.116**
2. Sang-Ngoen T, Czumbel LM, **Sadaeng W**, Miko A, Nemeth DI, Matrai P, Hegyi P, Toth B, Csupor D, Kiss I, Szabo A, Gerber G, Varga G, Keremi B. (2021). Orally Administered Probiotics Decrease Aggregatibacter actinomycetemcomitans but Not Other Periodontal Pathogenic Bacteria Counts in the Oral Cavity: A Systematic Review and Meta-Analysis. Front Pharmacol, 12:682656: 1-14. **IF: 5.881**