## OP-031 [Apitherapy]
## Development of Nanoemulsion-Based Water-Soluble Propolis (N-WEEP) Technology for Oral Anti-Inflammation and Gum Health

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The increasing prevalence and economic burden of gingivitis and periodontal diseases highlight the urgent need for effective therapeutic strategies. Despite the rising incidence of oral health-related conditions worldwide, there remains a lack of definitive treatments for oral inflammatory diseases and gum health, underscoring the necessity for innovative solutions.

Propolis, a bioactive natural compound known for its antioxidant, anti-inflammatory, and antimicrobial properties, contains key active constituents such as Artepillin-C, CAPE (Caffeic Acid Phenethyl Ester), Pinocembrin, and Galangin. These compounds make propolis a promising candidate for oral health applications. However, its poor water solubility and low bioavailability significantly limit its therapeutic potential.

This study aims to develop and evaluate a nanoemulsion-based water-soluble propolis complex to enhance its bioactivity and therapeutic efficacy for periodontal health. A stable nanoemulsion system was formulated using natural emulsifiers and polysaccharides, significantly improving the solubility and bioavailability of propolis and other bioactive compounds. The formulation incorporated plant-derived bioactive components and underwent high-energy emulsification, producing nanoparticles smaller than 100 nm to optimize absorption and functional performance.

The therapeutic potential of the nanoemulsified propolis complex was assessed by analyzing its effects on key inflammatory mediators involved in periodontal disease pathogenesis, including pro-inflammatory cytokines and bacterial lipopolysaccharides (LPS). Additionally, its antimicrobial activity was tested against major periodontal pathogens, including Porphyromonas gingivalis, Fusobacterium nucleatum, Tannerella forsythia, Treponema denticola, Prevotella intermedia, and Aggregatibacter actinomycetemcomitans.

Experimental findings demonstrated that the nanoemulsified propolis complex effectively inhibited key periodontal pathogens and modulated host inflammatory responses, suggesting its potential as a functional ingredient for periodontal health applications.

These results underscore the potential of nanoemulsion technology in enhancing the bioactivity and therapeutic efficacy of propolis. The findings contribute to the development of innovative functional foods and oral healthcare products, leveraging targeted antimicrobial, anti-inflammatory, antioxidant, and immune-modulatory mechanisms for the prevention and management of periodontal diseases.

# Innovative Water-soluble, Alcohol-Free Propolis Extract:

## Nanoemulsion Technology to Enhance Bioactivity for Gum Health & Periodontal Disease Prevention

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Spin-off Company of Korea Energy Research Institute

Seoul Propolis

# 1) Overview

Spin-off Company of Korea Energy Research Institute
Seoul Propolis

**Today**

- Seoul Propolis continues to innovate, introducing propolis into new market scenarios. We have established GMP and HACCP-certified factories for propolis production, and our dedication to quality and innovation is further evidenced by numerous patents and certifications, including HALAL and ISO 9001.

**2020s**

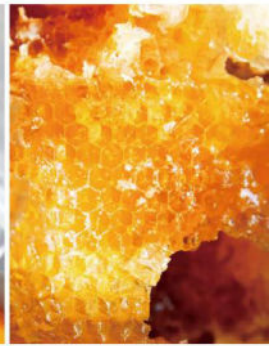
- Our research efforts continue to uncover the beneficial activities of propolis across various therapeutic areas, validated through clinical trials at major Korean research centers. Thanks to our unwavering commitment to R&D, Seoul Propolis' in-house laboratory has been recognized as an excellent corporate R&D center and a world-class product manufacturer by the Ministry of Science and ICT.

**2010s**

- Building on our innovative technology, Seoul Propolis diversified propolis extracts tailored to various applications while retaining the beneficial active compounds, including foods, beverages, pharmaceuticals, and cosmetics. We were honored with a gold medal for technology development at the 44th APIMONDIA (International Apicultural Congress).

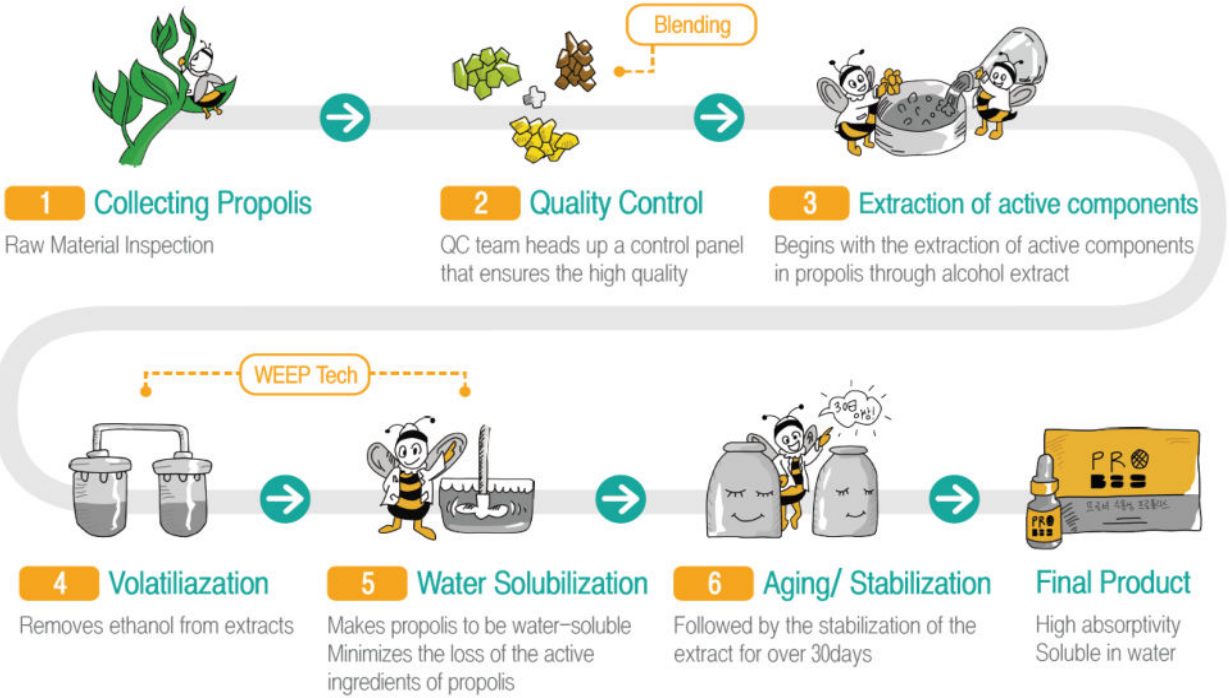
**2000s**

- Seoul Propolis was founded as a spin-off company of Korea Atomic Energy Research Institute, focusing on research and development in the early years. We developed the patented technology of 'Manufacturing Method of Water Soluble Propolis' in 2003.



# 2) Propolis and WEEP Technology

- Well known for antibacterial and antioxidant effects.
- However, poor solubility limits its applications.

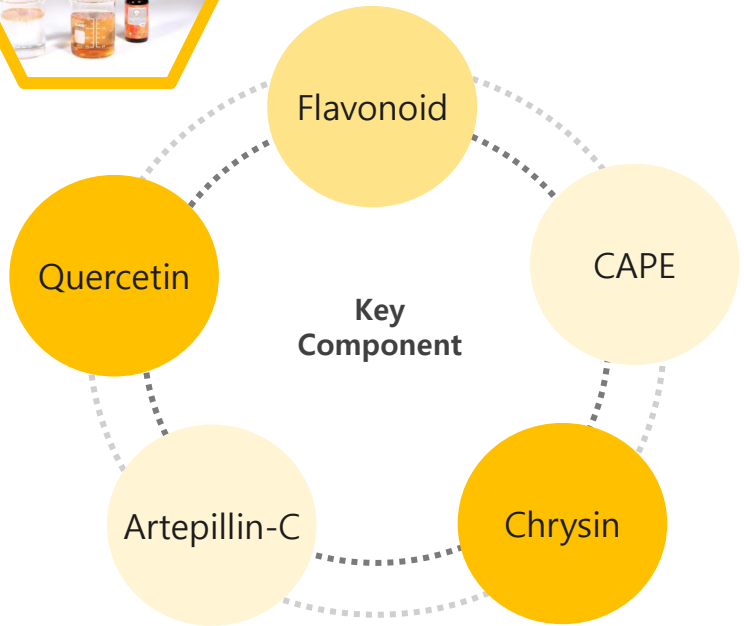


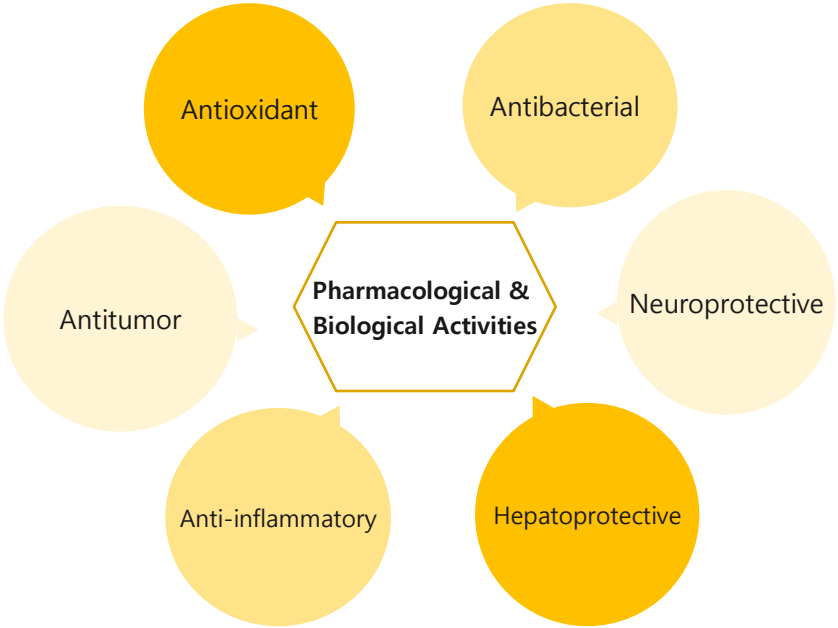
Ethanol Extracted Propolis

Alcohol Free and
Water-soluble Propolis

# 1) Propolis and its Compounds

- Propolis is a resinous mixture collected by honey bees from plant sources
- Its composition varies by origin, with 〉300 compounds identified



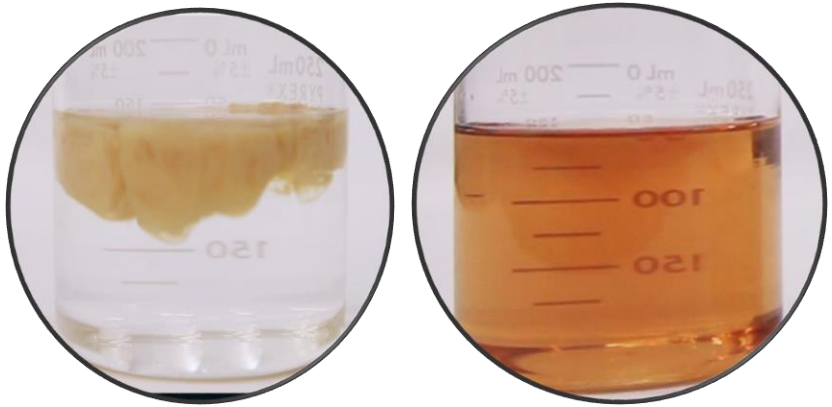



# 2) Propolis and its Compounds by Origins

| | Origin | Plant | Main Components |
|---|---|---|---|
| **Poplar** | Europe, North America, Asia, Australia | *Populus spp. of section Aigeiros Most often P. nigra L.* | Flavones, Flavanone, cinnamic acid and their esters |
| **Green** | Brazil | *Baccharis spp.,* | prenylated ρ-coumaric acid, Artepillin-C, diterpenic acid |
| **Birch** | Russia | *Betula verrucosa Ehrh.* | Flavones and flavonol(not the same as in Poplar type) |
| **Red** | Cuba, Brazil | *Dalbergia spp.* | Isoflavonoids(isovlavans, pterocarpans) |
| **Mediterranean** | Sicily, Greece, Croatia | *Cupressaceae(species unidentified)* | Diterpenes(mainly acid of labdane type) |
| **Clusia** | Cuba, Venezuela | *Clusia spp.* | Polyprenylated benzophenones |
| **Pacific** | Okinawa, Taiwan, Indonesia | *Macaranga tanarius* | C-prenyl-flavanones |
| **Korean Red Pine** | South Korea | *Pinus densiflora Sieb.* | Galangin, Chrysin |

# 1) Preparation of N-WEEP®

- Propolis extracts (different origins) + Green tea + Lecithin → Nanoemulsion
- Stabilized by WEEP® technology
- Micro-filtered → Clear, alcohol-free, water-soluble extract
- Enhanced bioavailability

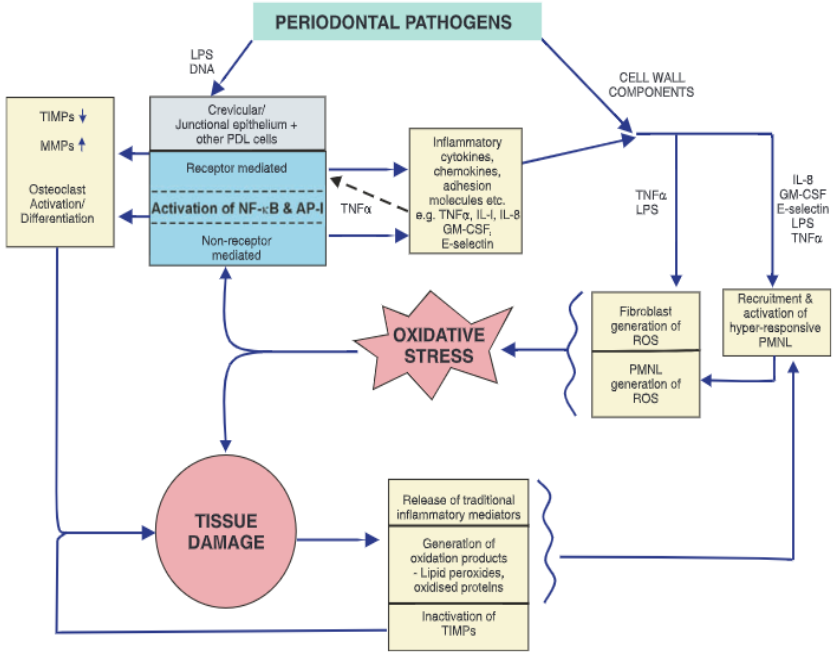


Ethanol Extracted WEEP Extract

| | WEEP Method | Ethanol Extracted |
|---|---|---|
| Extraction Method | Water-soluble, Alcohol-Free | Ethanol |
| Applications | Broad | Limited |
| Particle Size | <100 nm | 120~325 nm |
| Stability | Stable | Precipitation |
| Active Ingredients | Broad | Limited. |
| Alcohol Residue | ≤0.5% | >40% |

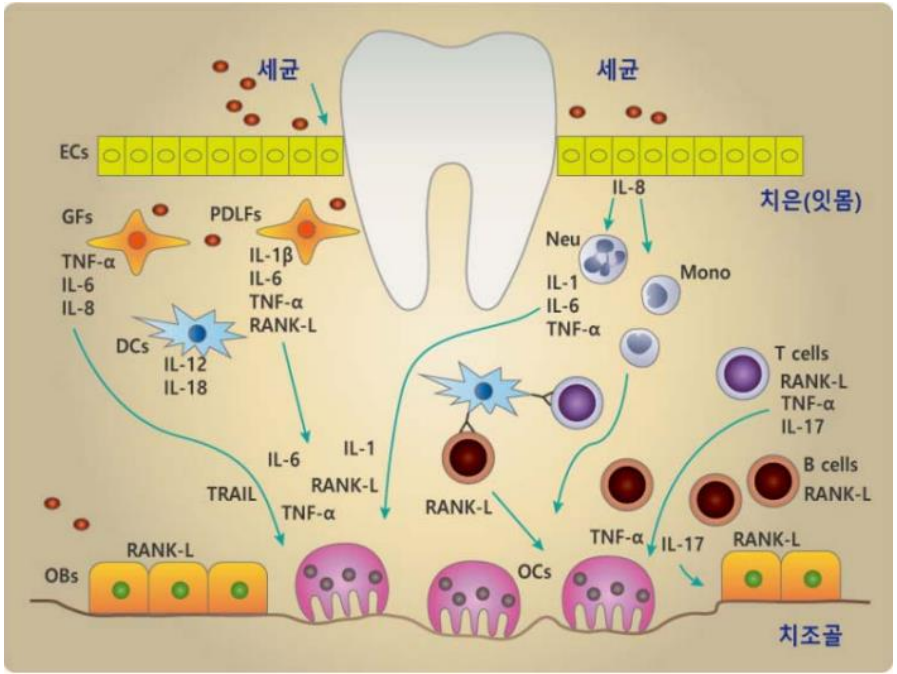
# 2) Mechanism of Periodontal Disease

- **Periodontal pathogens trigger inflammation and oxidative stress, leading to gum tissue damage**




Gum disease and antioxidants

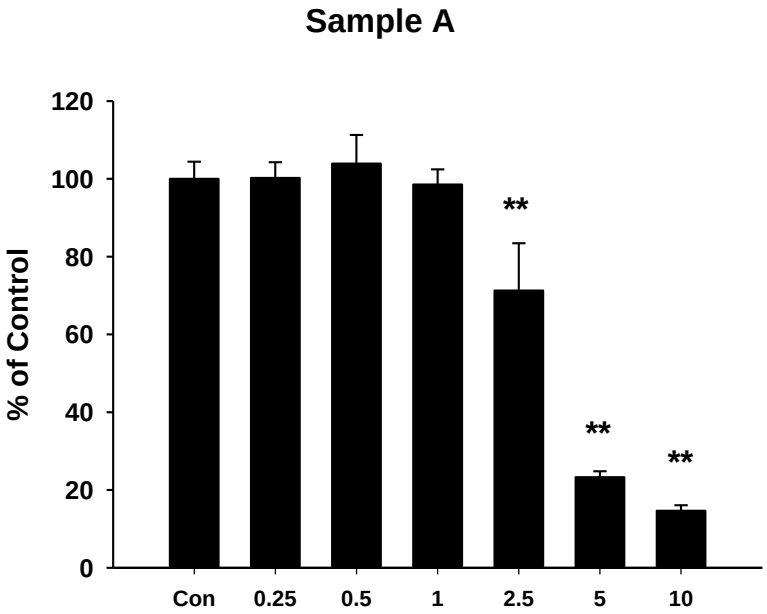
Periodontology 2000. 2007; 43(1): 160-232

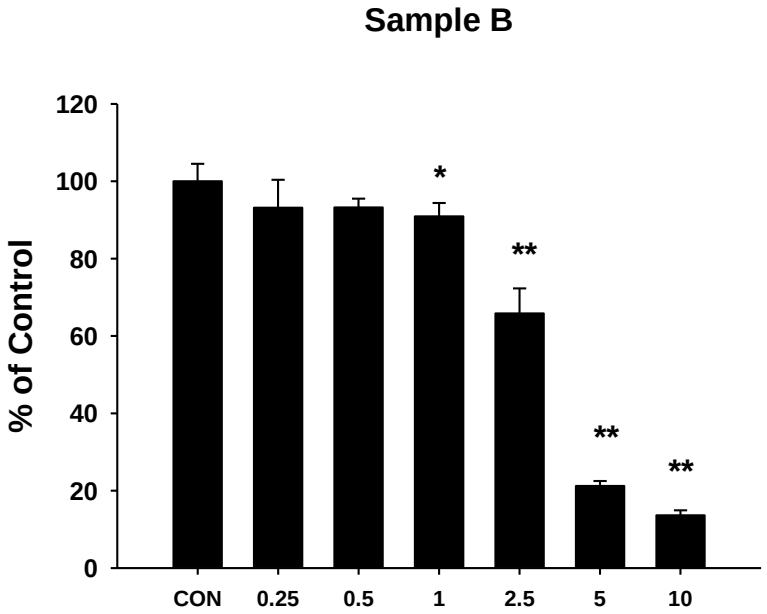



Gum Disease and Cytokines

Journal of Immunology Research, 2013

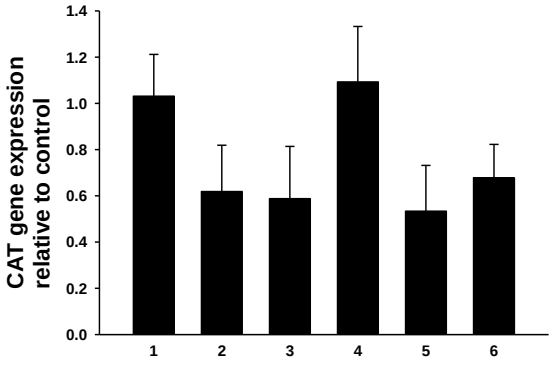
# 3) Cytotoxicity Test

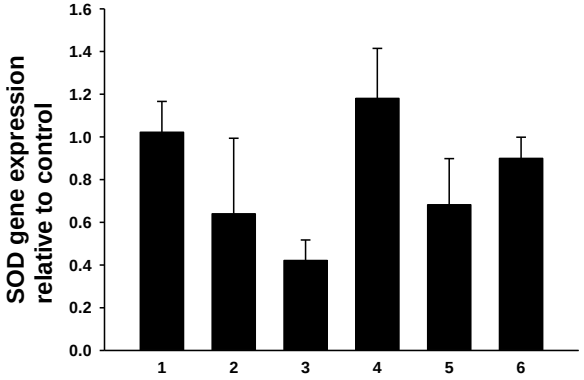


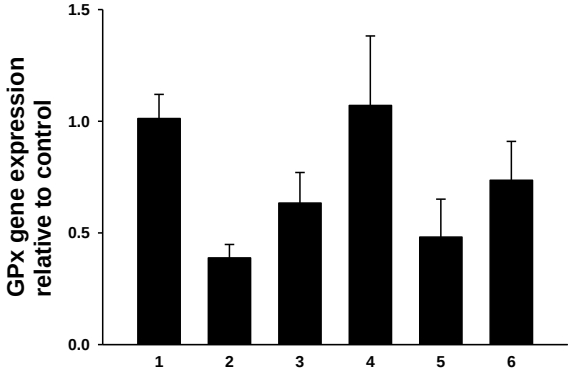




- Cytotoxicity above 2.5 μg/ml
- Safe range: 0.5–1 μg/ml (used for antioxidant & anti-inflammatory tests)

# 4) Antioxidant Activity



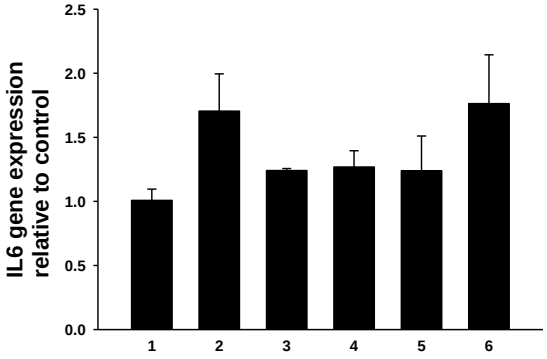



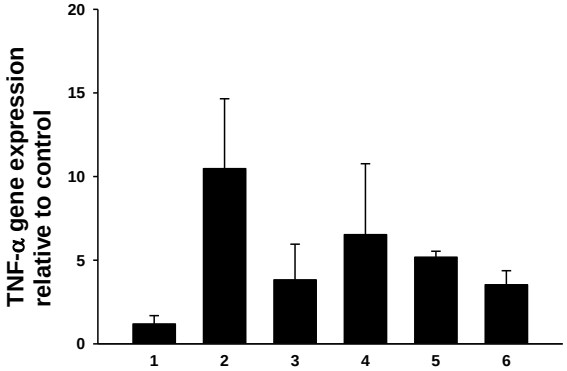



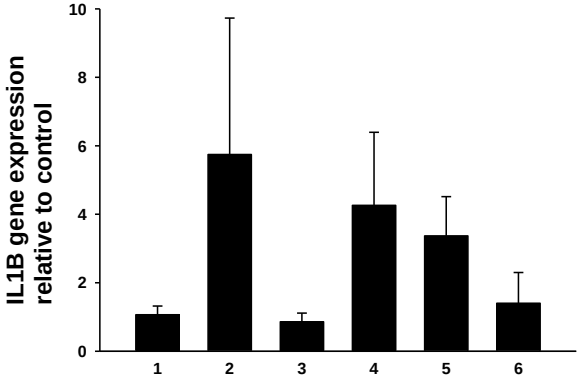

1. Control
2. $H_2O_2$
3. $H_2O_2$+A (0.5ug/ml)
4. $H_2O_2$+A (1 ug/ml)
5. $H_2O_2$ +B (0.5ug/ml)
6. $H_2O_2$ +B (1ug/ml)

- Sample A (1 μg/ml) significantly increased CAT, SOD, GPx expression under oxidative stress

# 5) Anti-inflammatory Activity




1. Control
2. $H_2O_2$
3. $H_2O_2$+A (0.5ug/ml)
4. $H_2O_2$+A (1 ug/ml)
5. $H_2O_2$ +B (0.5ug/ml)
6. $H_2O_2$ +B (1ug/ml)

- Sample A reduced IL-6; both A & B reduced TNF-α and IL-1β. Additional tests ongoing for IL-1β due to variation.

# 6) Pathogenic complexes of oral bacteria in periodontal disease

- **Red complex :** Key pathogens driving periodontitis progression

  *P. gingivalis, T. forsythia, T. denticola*

- **Orange complex :** Bridge bacteria, enhancing inflammation & synergy with Red

  *F. nucleatum, P. intermedia, C. gracilis, C. rectus, P. nigrescens, P. micra*

- **Green complex :** Associated with aggressive periodontitis

  *A. actinomycetemcomitans, E. corrodens*

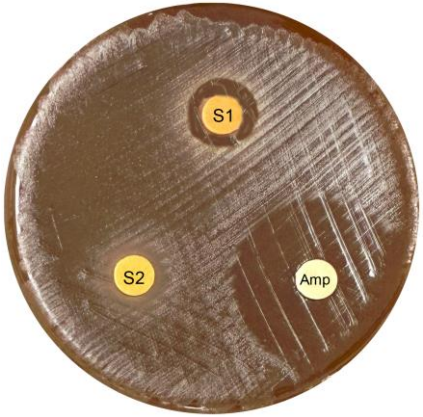
- **Yellow complex :** Early colonizers in dental biofilm

  *Streptococcus spp.*

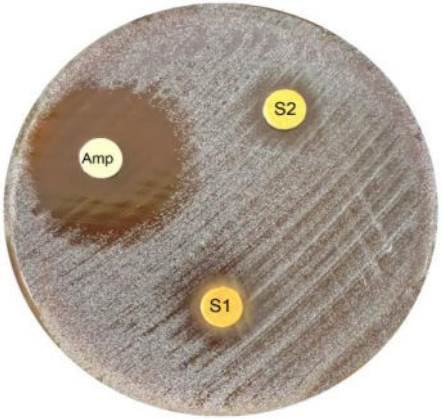
- **Purple complex:** econdary colonizers, low virulence

  *A. odontolyticus, V. parvula*

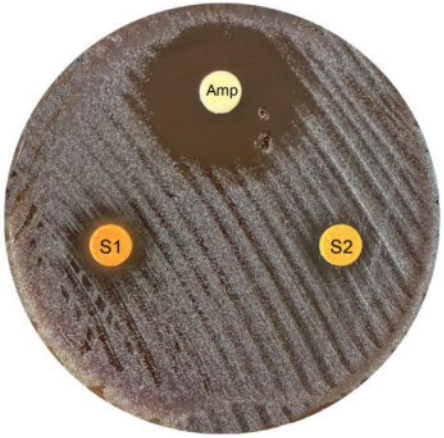
# 7) Antimicrobial Test : Disc Diffusion Method



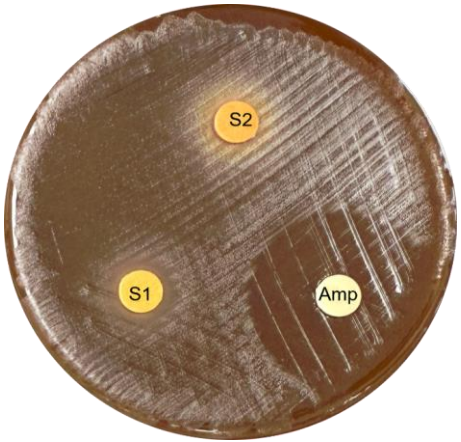

**Red complex**
*P. gingivalis*




**Orange complex**
*P. intermedia*

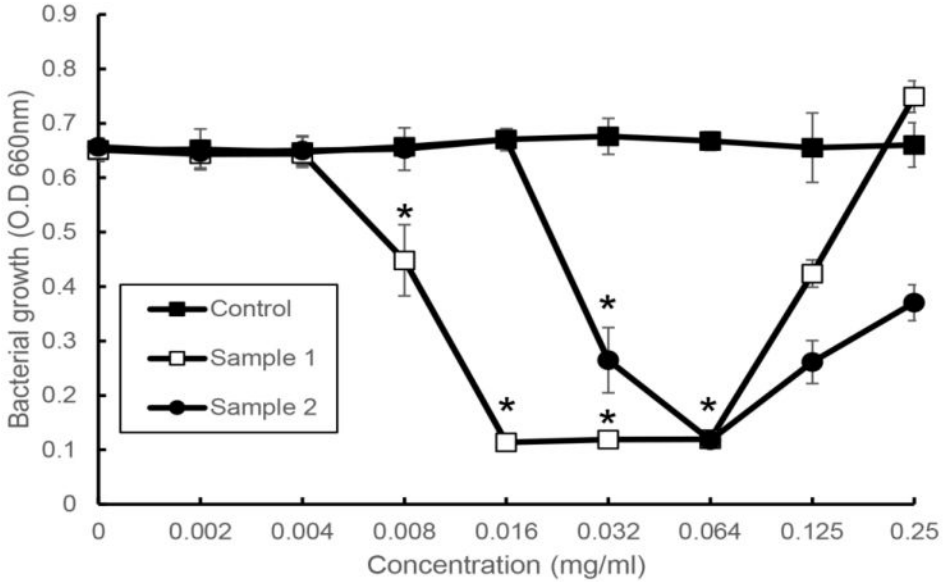



**Orange complex**
*F. nucleatum*



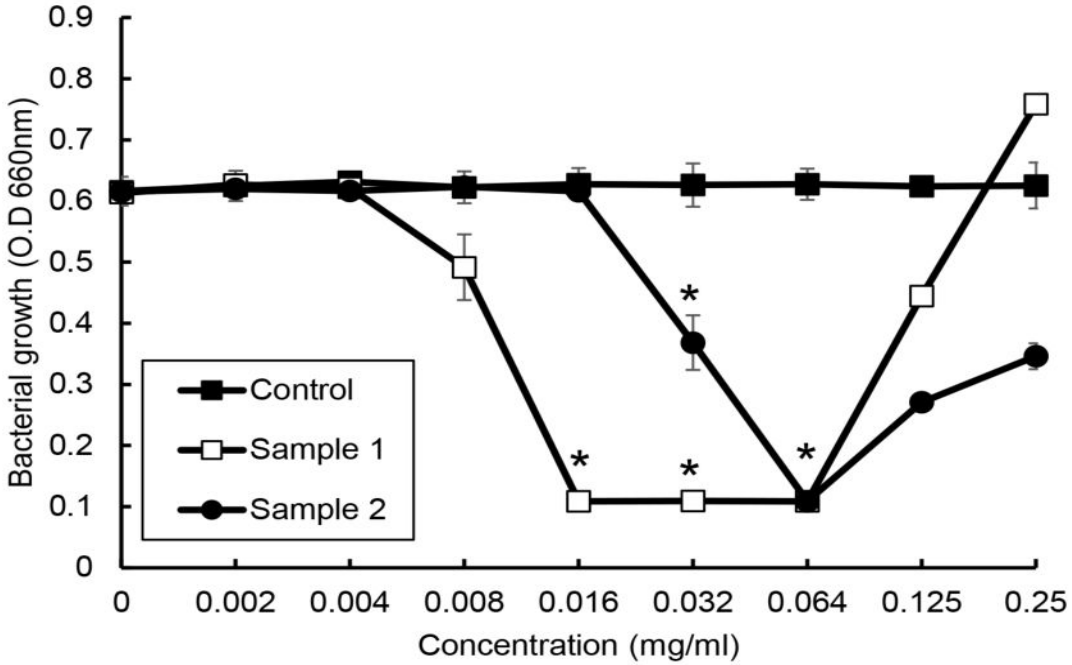

**Green complex**
*A. actinomycetemcomitans*

# 8) Antimicrobial Test for *P. gingivalis*



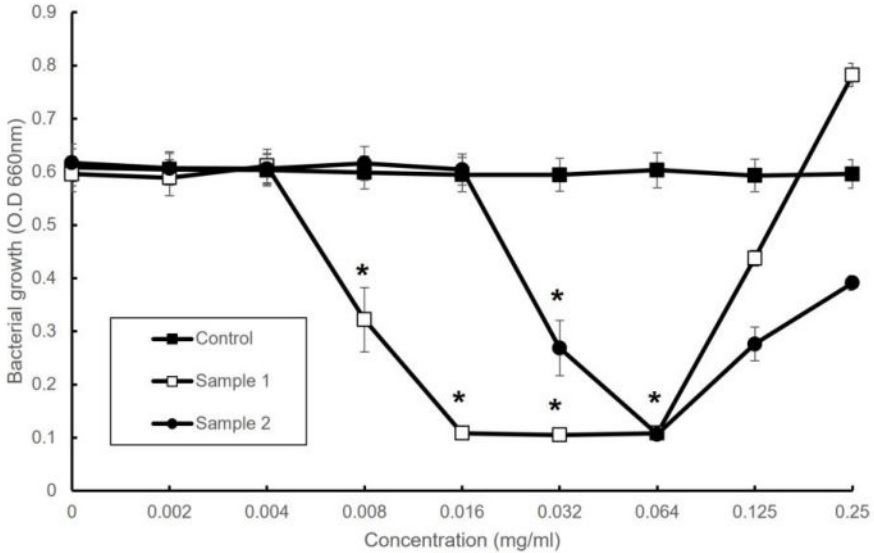

- Sample 1: Growth inhibited at 0.008 mg/ml, complete inhibition at 0.016 mg/ml
- Sample 2: Growth inhibited at 0.032 mg/ml, complete inhibition at 0.064 mg/ml
- ↑ Absorbance at ≥0.125 mg/ml due to color interference, not bacterial growth

# 9) Antimicrobial Test for *P. intermedia*



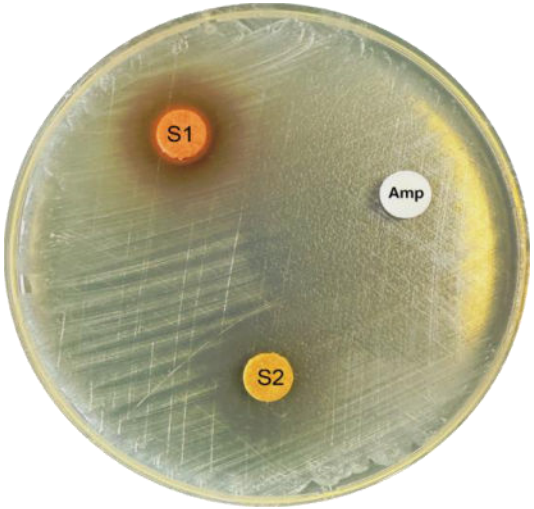

- Sample 1: Growth inhibited at 0.008 mg/ml, complete inhibition at 0.016 mg/ml
- Sample 2: Growth inhibited at 0.032 mg/ml, complete inhibition at 0.064 mg/ml

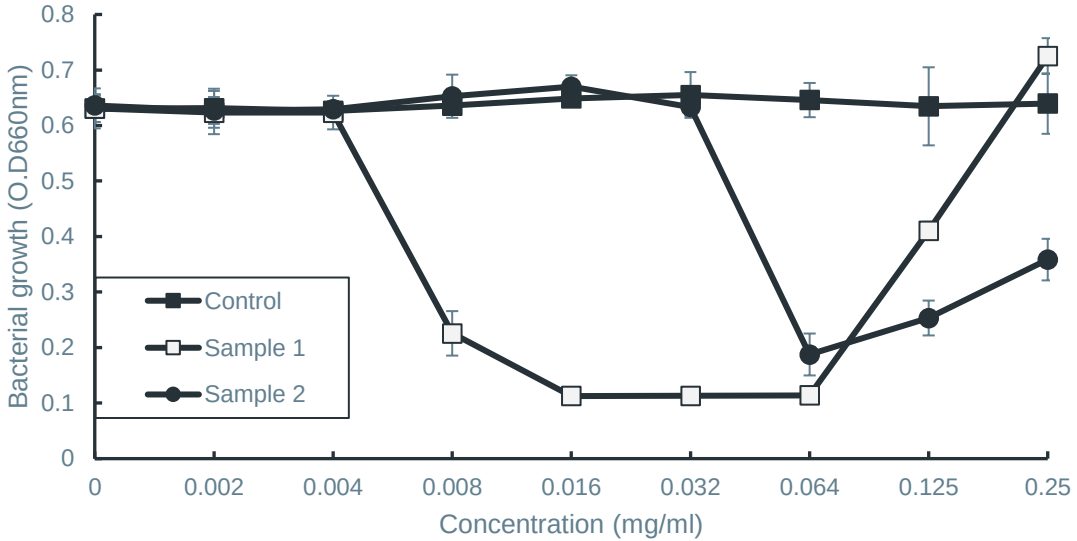
# 10) Antimicrobial Test for *A. actinomycetemcomitans*




- Sample 1: Inhibition at 0.008 mg/ml, complete inhibition at 0.016 mg/ml
- Sample 2: Inhibition at 0.032 mg/ml, complete inhibition at 0.064 mg/ml
- Sample 1 consistently more potent than Sample 2

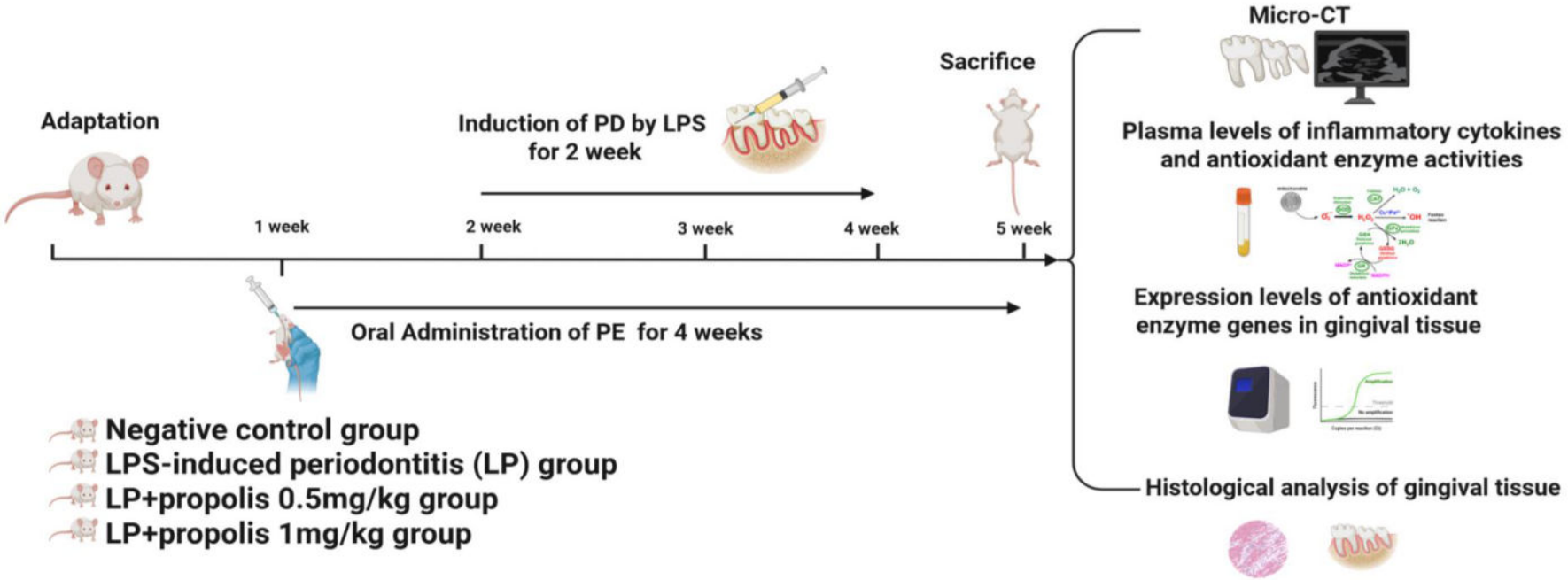
# 11) Antimicrobial Test for *S. mutans*



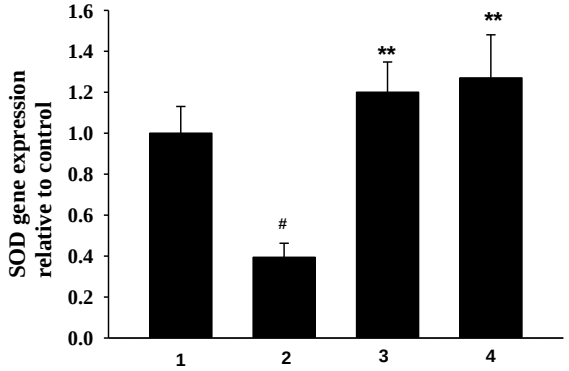

- Sample 1: Inhibition from 0.008 mg/ml, complete inhibition at 0.016 mg/ml
- Sample 2: Complete inhibition at 0.064 mg/ml
- Ampicillin used as control (positive reference)

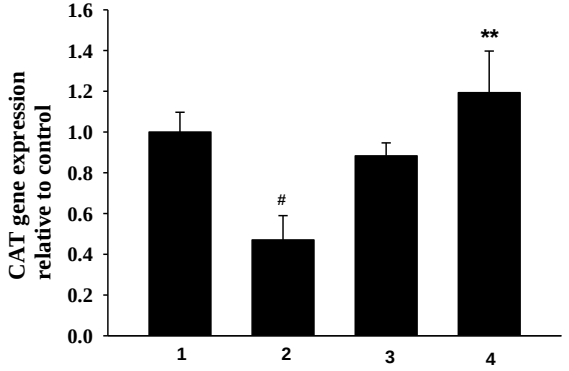
# 1) Effects of Propolis Using Animal Models of Periodontitis

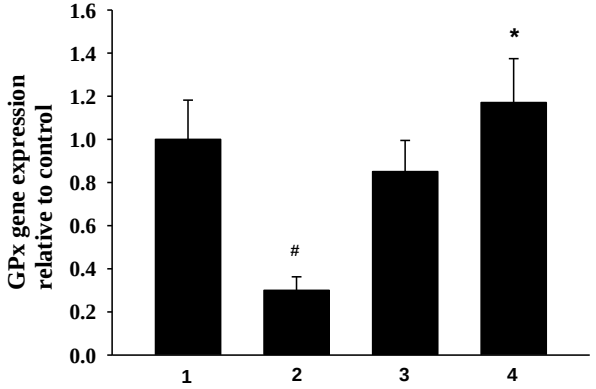



*Pre-clinical study performed independently by the Department of Dental Pharmacology, College of Dentistry, Dankook University (Seoul, Republic of Korea), in line with academic research purposes and not as part of EU cosmetic product safety assessment.*

# 2) Protective Effect of Propolis on CAT, SOD, and GPx Expression



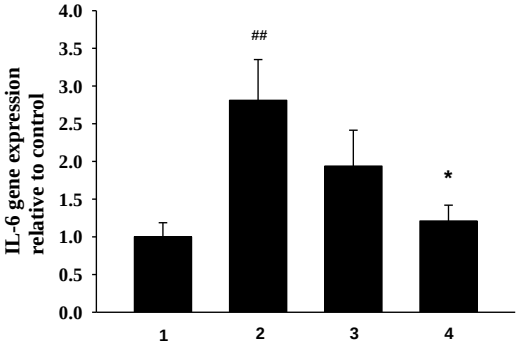



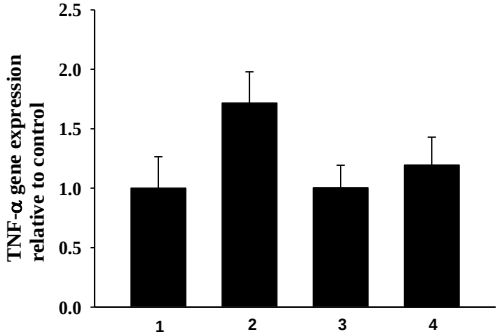



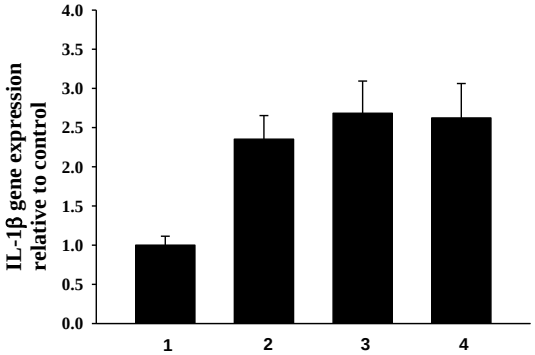

1. Control
2. Periodontitis
3. Periodontitis +Pro 0.5mg/kg
4. Periodontitis +Pro 1mg/kg

- Expression of CAT, SOD, and GPx genes was reduced in gingival tissue with periodontitis. Propolis administration at 1 mg/kg restored the gene expression of these antioxidant enzymes

# 3) Anti-inflammatory and Antioxidant Effects of Propolis in Periodontitis



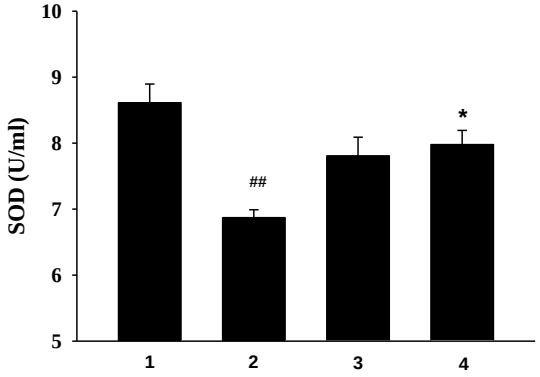



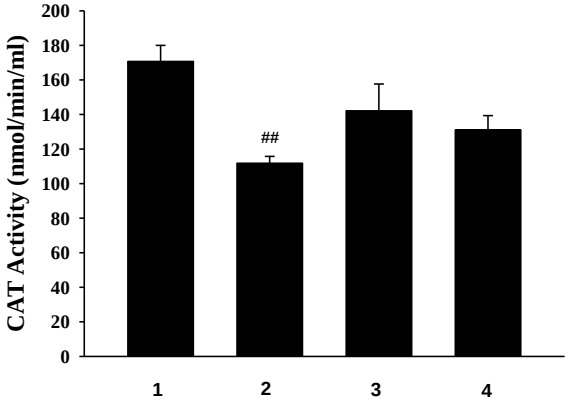



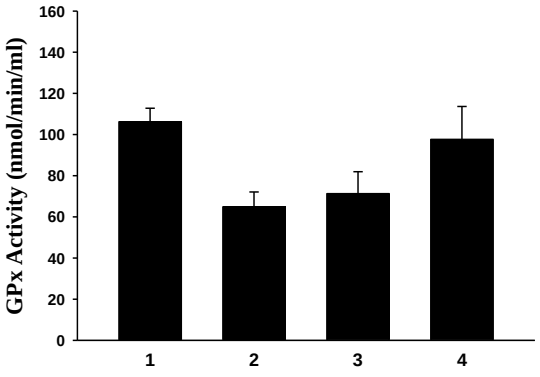

1. Control
2. Periodontitis
3. Periodontitis +Pro 0.5mg/kg
4. Periodontitis +Pro 1mg/kg

- IL-6: Significantly increased in periodontitis; significantly reduced by propolis.
- TNF-α: Showed a decreasing tendency with propolis, but not statistically significant.
- IL-1β: Also showed a decreasing tendency, but without statistical significance.

# 4) Propolis Enhances Antioxidant Enzyme Activity in Blood



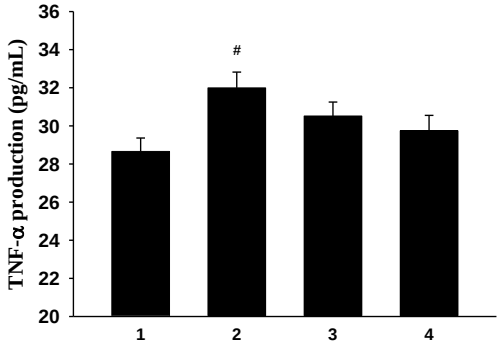



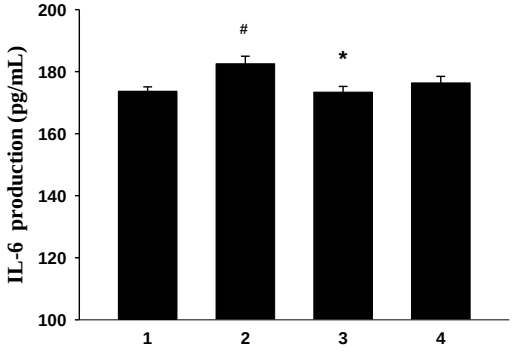



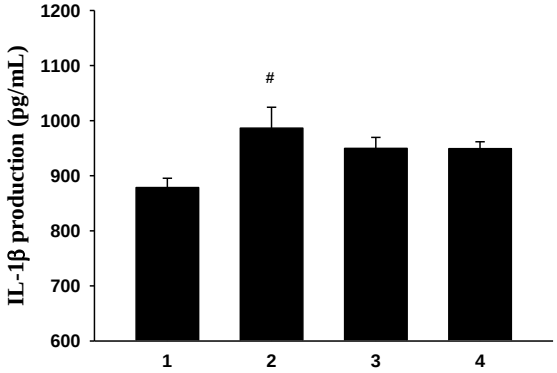

1. Control
2. Periodontitis
3. Periodontitis +Pro 0.5mg/kg
4. Periodontitis +Pro 1mg/kg

- SOD: Decreased in periodontitis; significantly increased by propolis.
- CAT: Significantly reduced in periodontitis; slightly increased by propolis.
- GPx: Decreased in periodontitis; decrease suppressed by propolis.

# 5) Propolis Reduces Inflammatory Cytokine Production in Blood




1. Control
2. Periodontitis
3. Periodontitis +Pro 0.5mg/kg
4. Periodontitis +Pro 1mg/kg

- TNF-α: Significantly increased in periodontitis; tended to decrease with propolis.
- IL-6: Significantly increased in periodontitis; significantly decreased with propolis (0.5 mg/kg).
- IL-1β: Significantly increased in periodontitis; slightly decreased with propolis.

# 1) Propolis (N-WEEP®) Improves Oral Health via Antioxidant, Anti-inflammatory, and Antibacterial Effects

**1. In vitro assay: Propolis Protects Against Oxidative Stress and Inflammation**

• Protects against $H_2O_2$-induced oxidative damage

• Restores antioxidant defense: ↑ CAT gene expression, Suppresses inflammation: ↓ IL-6 gene expression

• Antibacterial activity: Inhibits Red complex (3 types), Orange complex (2 types), and Green complex (1 type) pathogens

**2. In vivo assay : In Vivo Assay: Propolis Restores Antioxidant and Anti-inflammatory Balance**

- Restored gingival CAT, SOD, GPx gene expression reduced by periodontitis.
- suppressed IL-6 gene expression in gingival tissue.
- Increased blood SOD enzyme activity.
- Reduced blood IL-6 production

➢ **N-WEEP® Propolis supports gum health via antibacterial, antioxidant, and anti-inflammatory effects**

# 2) Propolis Across Diverse Product Categories

(Propolis Drinks)

(Capsule)

(Propolis Spray)

(Propolis Tincture)

(Animal Feed)

(Liquid Sachet)

(ODF)

(Mouthwash)

(Oral Spray)

(Toothpaste)

# 3) PROBEE: Science-driven Propolis for Wellness

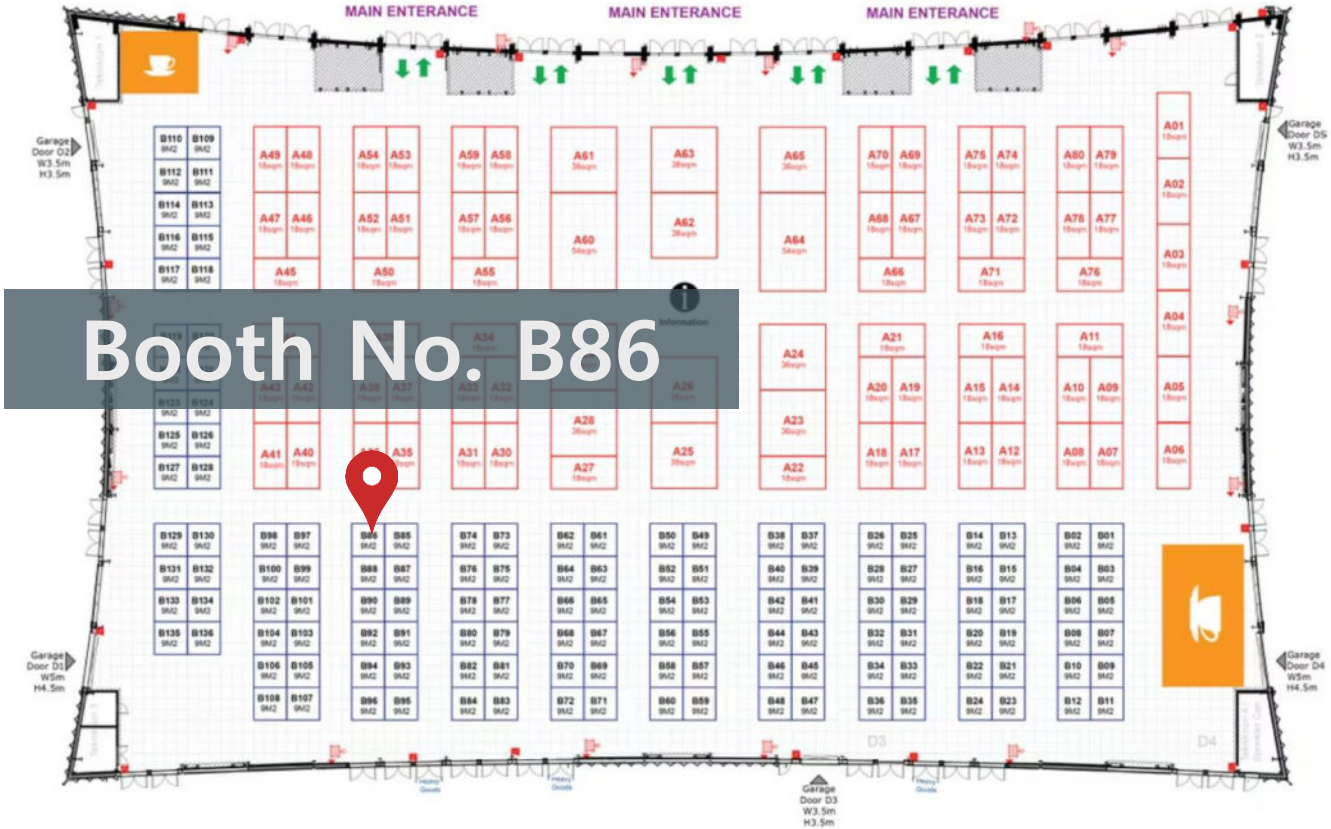
## Health Foods

## Personal Care Products

PROBEE

Filled with bee propolis

# 4) Booth Location



# Thank You

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