

High Performance Liquid Chromatography Analysis of the Active Ingredients and Evaluation of Anti-caries Potential of Thai Propolis Extracts

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ABSTRACT

Objective. This study aimed to determine and quantify the presence of the active components in Thai propolis extracts using high performance liquid chromatography (HPLC). Moreover, the anti-caries potential of Thai propolis extract and its active ingredients were tested.

Methods. Fifty milligrams of Thai propolis were extracted using either 100%, 90%, 80%, or 70% ethanol and subsequently analyzed using HPLC with a mobile phase gradient system of 10-100% acetonitrile in 0.05% aqueous ortho-phosphoric acid, flow rate of 0.8 mL/min, and detection wavelength of 280 nm. Varying concentrations of Thai propolis extracts as well as four active ingredients were subjected to agar well diffusion test against the growth of *Streptococcus mutans* (*S. mutans*) or *Lactobacillus casei* (*L. casei*).

Results. The concentrations of the four active ingredients: vicenin-2, vitexin, apigenin, and cinnamic acid, were significantly affected by ethanolic concentrations. The chromatographic peaks of all active ingredients from 70% and 80% ethanolic extracts appeared more defined, as compared to those which used higher concentrations of ethanol for extraction. Except for the absolute ethanolic extract, all of the examined propolis extracts, as well as its active ingredients inhibited both *S. mutans* and *L. casei*.

Conclusions. Thai propolis extracts contain vicenin-2, vitexin, apigenin, and cinnamic acid as part of its active ingredients. These were found to be significantly affected by the increase in ethanol during its extraction. The presence of these active ingredients might have contributed to the anti-caries potential of Thai propolis extracts.

Keywords: antibacterial activity, high performance liquid chromatography, flavonoids, phenolic compounds, Thai propolis extracts



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INTRODUCTION

Propolis is a natural, non-toxic resinous substance obtained by honeybees from different plant exudates.^{1,2} It is primarily used by the bees to seal holes in their honeycombs, smooth out the internal walls, and protect their habitat from intruders.¹ Propolis from various parts of the world such as Europe, America, Asia including different regions of Thailand has been the subject of various studies such as medicine, dentistry, cosmetics, and food industry.³ An alcoholic fraction of propolis sourced from Nan province, Thailand, has elucidated the significant antibacterial activities against the growth of Gram-positive (*Staphylococcus aureus*, *Paenibacillus larvae*) and Gram-negative (*Escherichia coli*) bacterial species, when compared with methanol, hexane, and dichloromethane extracts.⁴ Upon analysis of its chemical structure, it revealed that the said propolis' major active ingredient are phenolic compounds.⁴

Apart from the general systemic health benefits afforded by propolis extracts, numerous analyses which focused on its impact on dental treatment and prevention have been documented. Chailertvanitkul et al. demonstrated that Thai propolis extract, when formulated as a pulp-capping agent, can inhibit *Streptococcus mutans* (*S. mutans*) and *Lactobacillus casei* (*L. casei*), without causing any toxicity to dental pulp cells.⁵ This was further supported by another study which investigated the effect of a Thai propolis product on mechanically-exposed dental pulps of New Zealand white rabbits.⁶ Its findings showed that reparative dentin bridge formation was more orderly when using a Thai propolis as pulp capping material, when compared to those pulps capped with calcium hydroxide.⁶

Thai propolis extracts also have preservative and proliferative effects on avulsed teeth. It was revealed that 2.5 mg/mL is the most effective dose for preserving the viability of human periodontal ligament cells, comparable to Hank's balanced salt solution.⁷ A study of Thai propolis extract on periostin and S100A4 mRNA expression in human periodontal ligament cells in avulsed teeth also showed that Thai propolis extract was able to preserve periostin mRNA levels, and the expression of S100A4 mRNA was reduced, thus maintaining PDL cells' phenotype.⁸

Analysis of crude components of propolis showed that it consists of 50% resin, 30% wax, 10% essential oils, 5% pollen, and 5% other chemicals.^{1,3} However, propolis itself cannot be used for biological and pharmaceutical purposes due to its composition. Its extract must first be obtained either through pure water, ethanol, methanol, hexane, acetone, and / or chloroform extraction.⁹ The extraction of the active components of propolis mainly aims to eliminate the wax which is an ineffective part most often present in propolis.¹⁰ Numerous studies have documented that ethanolic solvents are able to isolate more phenolic compounds from propolis, and its extracts have higher antioxidant activity.¹¹ However, there have been conflicting findings as to which

ethanolic concentration yields the most profound outcome of the extraction procedures.

Wozniak et al. has compared between Poland propolis extracts obtained from either 70% or 96% ethanolic concentration. Qualitative and quantitative analysis of both extracts revealed that an extraction with 70% ethanol yielded higher amount of phenolic compounds.¹² A prior study comparing Thai propolis extracts obtained using 30%, 40%, 50%, and 70% ethanol showed that the phenolic content and anti-oxidant properties were directly proportional to the amount of ethanolic concentration used during extraction.¹³ These findings corroborate the finding of Gomez-Caravaca et al. which reported that ethanol extraction of propolis is most suitable in obtaining its polyphenolic components, and that 70-80% concentration of ethanol is commonly used.⁹

The major active components of propolis have been proven to be relative to the type of vegetation and temperature in the area where the sample was obtained, variations in the queen bee, and seasonality during the time of sample collection.^{2,14} The active ingredients of propolis tend to reflect composition of predominant vegetation and be affected by the season at the location where the bees have foraged. Moreover, differing bee species would also have an impact on the chemical composition and propolis quality.¹⁵ One of the most prevalent active ingredients in propolis extracts are flavonoids and phenolic acids owing to their abundance and biological activity.¹⁶ Over 300 chemical components belonging to flavonoids, phenolics, and terpenes have been identified in propolis extracts.^{16,17} High Performance Liquid Chromatography (HPLC) analysis of Brazilian propolis extracts showed that it is comprised mainly of apigenin, quercetin, and kaempferol. These findings were further supported by Righi et al. which identified vicenin-2 and apigenin as some of its flavonoid contents through gas chromatography-mass spectrometry.¹⁸ Propolis obtained from China were identified to contain vitexin, a flavonoid which has a significant role in reducing the lipopolysaccharide-induced cytokine expression in human dental pulp stem cells.¹⁹ Whereas, propolis from Lithuania have high amounts of vitexin and apigenin, which are credited for its anti-oxidant activity.²⁰

Numerous researches have proven that synergism between different compounds allow propolis to exert its positive pharmaceutical and biological activities.^{15,16,21,22} Another key ingredient that have been highlighted by numerous studies are phenolic acids like cinnamic acid.¹⁶ Ikeno et al. have documented cinnamic acid as one of the major components of propolis from Japan and China.²³ When tested against cariogenic bacteria such as *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus cricetus*, these propolis samples were able to inhibit its glucosyl transferase as remarkable as 60%.²³ In addition, Koo et al. documented that ethanolic extracts of propolis from Southern Brazil were capable of preventing smooth surface and sulcular caries when applied to desalivated rats orally infected with *Streptococcus sobrinus*.²⁴

However, propolis harvested from different countries and locations may have different predominant active ingredients; and congruently, may also have different antimicrobial properties against cariogenic bacterial species. Therefore, this study aimed to determine and quantify the presence of flavonoids, such as vicenin-2, vitexin, apigenin, and phenolic acids, such as cinnamic acid, in Thai propolis extracts using HPLC; and to analyze whether its yield was affected by the ethanolic concentration used in extraction. The antimicrobial effects of these active components were also investigated.

MATERIALS AND METHODS

Preparation of Thai Propolis Extract

Propolis samples were obtained during summer (May to July 2021) from an apiary in Nong Khai province, North-eastern Thailand which had longan trees (*Dimocarpus longan*) as the predominant vegetation. The samples were stored and kept refrigerated at 4°C before use. Fifty milligrams of propolis were placed separately in microcentrifuge tubes and added with 0.5 mL of either 100%, 90%, 80% or 70% of ethanol. The samples were vortexed, followed by ultrasonic-assisted extraction at 60 Hertz for 20 minutes at ambient temperature. The tubes were centrifuged at 10,000 rpm for 5 minutes and the supernatant was collected in a new tube. The extraction process was repeated three times. The supernatants were pooled and evaporated using a rotary evaporator overnight and redissolved in 1 mL ethanol for HPLC analysis. The resulting concentration for each extract that was used for the HPLC analysis and subsequent antimicrobial test was 50 mg/mL of ethanol.

HPLC Analysis

Analytical standards of the flavonoids, vicenin-2 and vitexin, were purchased from Biopurify Phytochemicals (Sichuan, China), while apigenin was obtained from Wako Pure Chemical Industries (Osaka, Japan). The standard for cinnamic acid was purchased from Merck Co. (Darmstadt, Germany). Analytical grade of methanol, acetonitrile, and 0.05% ortho-phosphoric acid were used during the HPLC analysis.

The HPLC system (Thermo Fisher Scientific, CA, USA) was performed using a ThermoFisher Spectra System machine with an RP-18 column (LichroCART, 250 mm x 4 mm, 5 µm particle size, Merck Germany). The resulting chromatogram was reviewed and analyzed using the accompanying ChromeQuest computer system. The HPLC system used was adapted and modified from an earlier study by Choonong et al.²⁵ The solvent used and its concentrations were modified to become ortho-phosphoric acid so that it could better interact with the polar components of the active ingredients. The mobile phase system started at 10% acetonitrile (A) in 0.05% aqueous ortho-phosphoric acid (B) which was held for 5 minutes. The gradient elution

proceeded as follows; 5% increment of A every 5 minutes until it reached 30% at 25-30 minutes, followed by 50% A at 30-35 minutes, 70% A at 35-40 minutes, 100% A at 40-45 minutes, and 10% A at 45-50 minutes. The flow rate was set at 0.8 mL/min for the whole duration of run time, while the wavelength was set at 280 nm. The samples were injected one-by-one into the system at a volume of 20 µL per injection using an autosampler. The peak area and chromatogram of each sample were recorded and analyzed.

To establish the validity of the system used, analytical standards of vicenin-2, vitexin, apigenin, and cinnamic acid were mixed with ethanol to obtain concentrations of 50.0, 25.0, 12.50, 6.25, 3.13, 1.56, and 0.78 µg/mL for all compounds. The linearity of the standard curve produced by the chromatographic conditions was assessed by plotting the peak area of each standard solution versus the concentration. The correlation coefficient and the y-intercept of the linear regression line were examined using Microsoft Excel 2010.

Intra-day precision was evaluated on the same day as the calibration curve by injecting 20 µL of each concentration of standard solution in triplicate. Whereas, inter-day precision was determined over three days of analysis by replicating the injection of each concentration of standard solutions each day. Data on intra-day precision and inter-day precision were analyzed in terms of its relative standard deviation (RSD).

To determine the limit of detection, a standard solution with the concentration close to the detection limit was injected thrice. The signal height and baseline noise were averaged. Moreover, in identifying the limit of quantification (LOQ), six standard solutions with the amounts in the range from the expected LOQ up to 20 times this amount were injected. All samples were injected 6 times and the standard deviation for each amount were calculated. These methods are in accordance with the guidelines set by International Conference on Harmonization Guidelines.

Antimicrobial Activity of Propolis and its Bioactive Components

Upon receiving Ethics Review Exemption (HE 642173) from the Research Ethics Board in accord with the guidelines set by Khon Kaen University, Thailand, the antimicrobial activity of Thai propolis extracts and its active ingredients were analyzed. 100%, 90%, 80%, and 70% of Thai propolis extract, along with the analytical standards of the four active ingredients were tested based on its inhibitory capacity against *S. mutans* and *L. casei* using agar well diffusion experiment. The concentration of active ingredients used was approximate to the concentration obtained (in µg/mL) during HPLC analysis, specifically: 50 µg/mL vicenin-2, 50 µg/mL vitexin, 100 µg/mL apigenin, and 40 µg/mL cinnamic acid. Bacterial cultures of *S. mutans* (DMST 8777; Department of Medical Science, Ministry of Public Health, Bangkok, Thailand) and *L. casei* (TBRC 388; National Science and Technology Development Agency, Bangkok, Thailand) were inoculated in sterile BHI (HiMedia Laboratories, India)

and MRS (HiMedia Laboratories, India) broths, respectively. Bacterial cultures were incubated overnight at 37°C in 5% CO₂ condition for 24 hours. A concentration of 10⁵ colonies per mL from each bacterial broth was prepared for an inoculation on the plates using cotton swab. Upon drying, a sterile borer was used to create 3-4 wells of 6 mm diameter x 6 mm height on each agar plate. Subsequently, aliquots of 50 µL of each of the following solution were placed on the assigned agar well: 100% ethanolic extract, 90% ethanolic extract, 80% ethanolic extract, 70% ethanolic extract, 50 µg/mL vicenin-2, 50 µg/mL vitexin, 100 µg/mL apigenin, and 50 µg/mL cinnamic acid. Both 2% chlorhexidine (CHX) and 2.5% sodium hypochlorite (NaOCl) have been documented as effective antibacterial solutions in dentistry, thereby being used as positive control groups.^{26,27} Normal saline solution served as a negative control. Each setup was repeated three times in triplicate and the plates were incubated for 48 hours at 37°C in 5% CO₂ condition prior to the measurement of the diameter of zone of inhibition around each well using a digital caliper.

Statistical Analysis

Statistical analysis was performed using SPSS software, version 28 (Chicago, IL, USA). To determine whether the changes in ethanolic concentrations used during its extraction have a significant effect on the concentration of vicenin-2, vitexin, cinnamic acid, and apigenin, a One-Way ANOVA was used, followed by Tukey's post-hoc test. To compare the antibacterial activity between the various ethanolic extracts, as well as between the active ingredients, Kruskal-Wallis test was used due to the non-normal distribution of the data, followed by Mann-Whitney post-hoc test. The level of significance for all analyses was set at $P < 0.05$.

RESULTS

HPLC Analysis of Active Ingredients of Thai Propolis Extracts

To aid in identifying the presence of the desired active ingredients, an analysis of just the analytical standards was performed using the HPLC system specified in Figure 1. It showed the retention time of the following active ingredients to be as follows: (1) vicenin-2: 11.99 mins, (2) vitexin: 18.74 mins, (3) apigenin: 32.64 mins, and (4) cinnamic acid: 33.77 mins.

The chromatograms obtained from the HPLC analysis of 100%, 90%, 80% and 70% extracts of Thai propolis showed that decreasing ethanolic concentration resulted in more profound peaks and higher concentrations for all the four active ingredients analyzed (Figure 2).

Among the ethanolic concentrations, 70% ethanolic extract had a significantly higher concentration of vicenin-2, vitexin, cinnamic acid, and apigenin ($P < 0.001$) (Figure 3).

By injecting at least six concentrations of the standard solutions, the linearity of the HPLC methods was ascertained.

A regression equation was formulated using the average area under the curve of each concentration. All four active ingredients showed good correlation coefficients, within the acceptable value of >0.99 . Along with the method's linearity, the limit of detection (LOD) and limit of quantification (LOQ) were also determined by calculating the average baseline noise with a signal 3 and 10 times higher than the said value (Tables 1 and 2), respectively. Analysis revealed that the limit of precision assessed by the relative standard deviation (RSD) was acceptable, suggesting the reliability of the aforementioned HPLC analysis results.

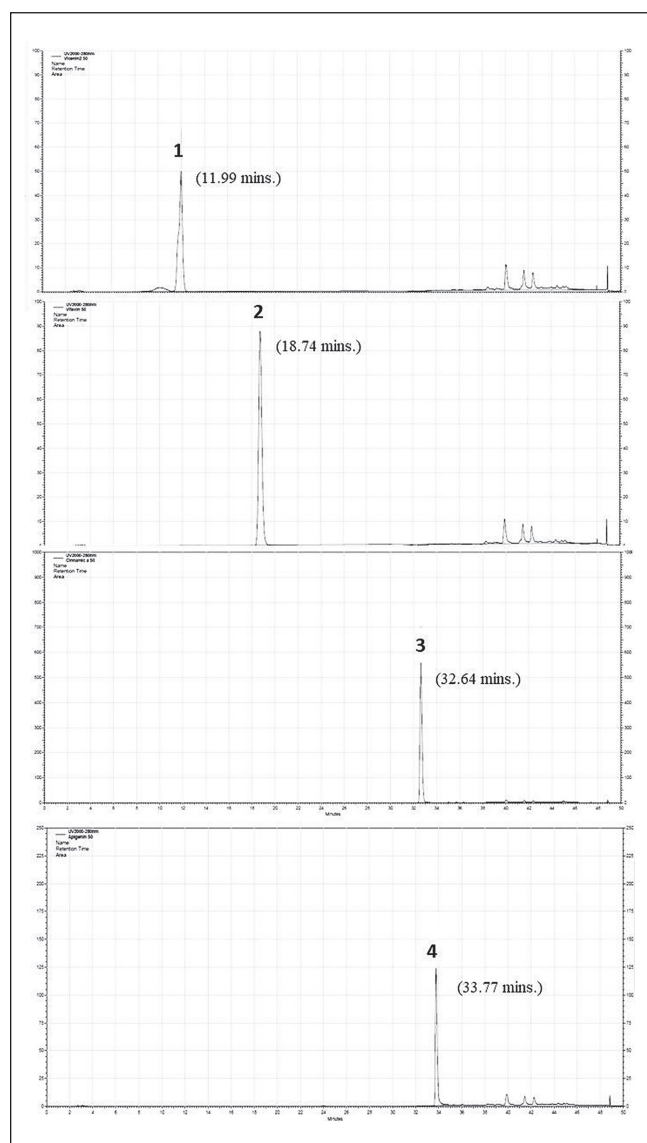


Figure 1. Retention time of analytical standards. 1 – vicenin-2, 2 – vitexin, 3 – apigenin, 4- cinnamic acid.

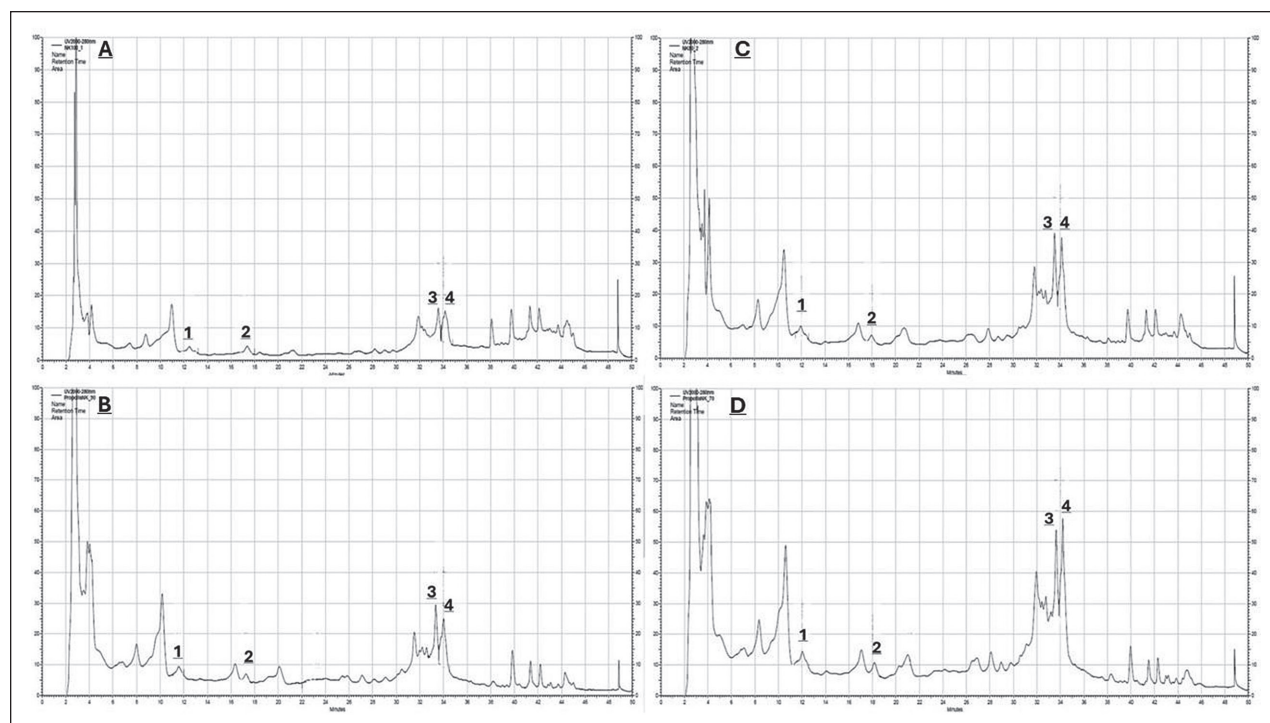


Figure 2. HPLC chromatograms of 100% (A), 90% (B), 80% (C), and 70% (D) extracts of Thai propolis. (1 – vicenin-2, 2 – vitexin, 3 – cinnamic acid, 4 – apigenin).

x-axis: retention time in minutes, y-axis: absorbance of compounds in mAU.

Antibacterial activity of Thai propolis extracts against the growth of *S. mutans* and *L. casei*

The absolute extract of Thai propolis yielded with no zones of inhibition for both *S. mutans* and *L. casei* plates (Figure 4). The zones of inhibition for Thai propolis extracts against both *S. mutans* and *L. casei* showed that 90%, 80%, and 70% ethanolic extracts had close values (Figures 4A and 4B). These mean zones of inhibition for the three extracts demonstrated no statistically significant difference between groups but were all significantly higher than 100% extract and the negative control group, as indicated by Kruskal-Wallis test with Mann-Whitney post-hoc analysis. The mean zones of inhibition from the positive controls, 2% CHX and 2.5% NaOCl, were significantly higher than all other solutions tested against *S. mutans* and *L. casei*. (Figure 4).

DISCUSSION

The antibacterial potential of the active ingredients identified from Thai propolis extracts was investigated using the standard solutions prepared at concentrations analogous to the concentrations obtained from 70% Thai propolis extract, which was the extract that recorded the highest yields from HPLC analysis. All four active ingredients showed an antibacterial activity against *S. mutans* and *L. casei* at close values, approximately 9 mm for both microorganisms. Upon analysis using Kruskal-Wallis test, the mean zones of

inhibition of all active ingredients versus *S. mutans* and *L. casei* showed no statistically significant difference (Figure 4).

Very few studies have delved into analyzing the active components of propolis through the use of HPLC. HPLC

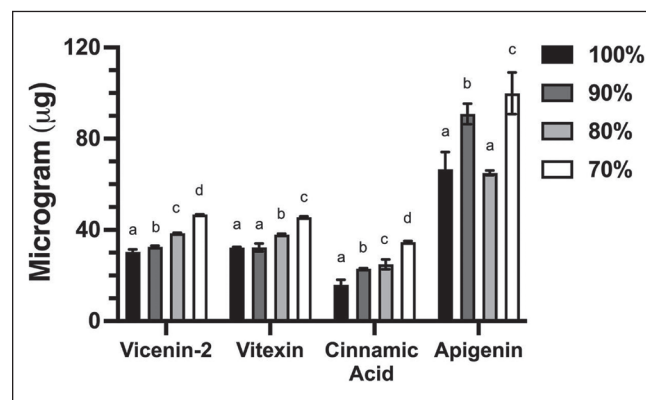


Figure 3. Equivalent concentrations of four active ingredients presented as mean $\pm$ standard deviation (in units of micrograms/gram dry weight of propolis). Each column of ethanolic extract of propolis (with different superscript letter within every active ingredient) represents a statistically significant difference as determined by One-Way ANOVA and Tukey's Post Hoc ($P < 0.05$).

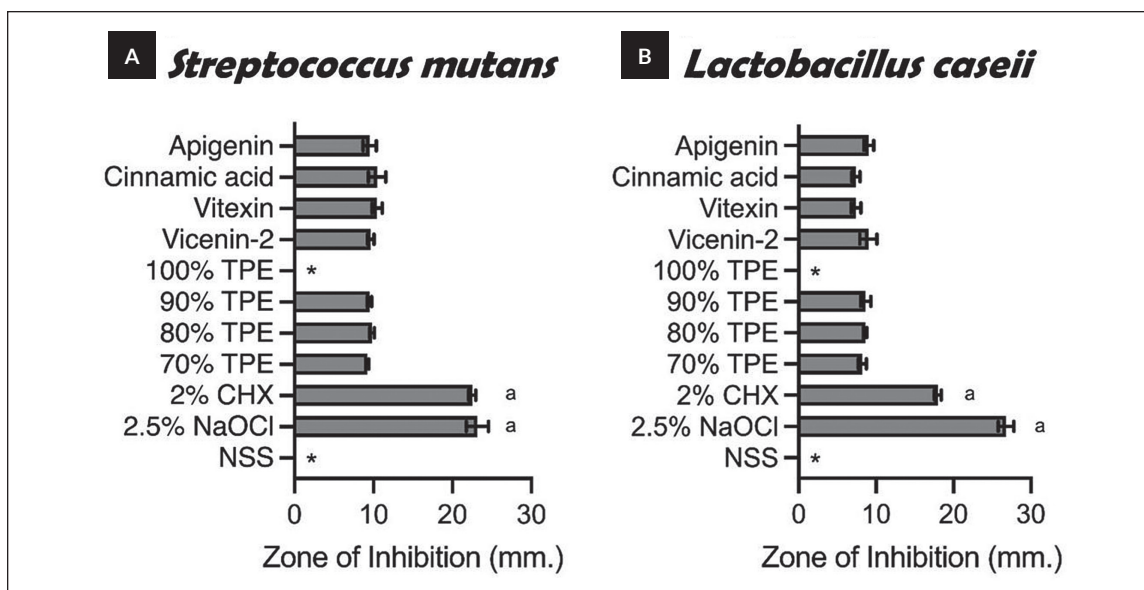


Figure 4. Antibacterial activity of ethanolic extracts of Thai propolis against the growth of (A) *S. mutans* and (B) *L. casei*, in comparison with four active ingredients. Asterisk (*) indicates a significant difference from other test groups; a – indicates a significant difference from 90%, 80%, and 70% extracts as determined by Kruskal-Wallis test and Mann-Whitney post-hoc analysis ($P < 0.05$).

TPE = Thai propolis extracts, CHX = Chlorhexidine, NaOCl = Sodium hypochlorite, NSS = Normal saline solution

Table 1. Regression Equation, Correlation Coefficients, Limit of Detection (LOD) and Limit of Quantification (LOQ) for the Active Components Determined by HPLC

Component	Assay Range ($\mu\text{g/mL}$)	Linear Equation	R^2	LOD	LOQ
Vicenin-2	0.78-50	$y = 22810x - 10140$	0.9997	0.193	0.645
Vitexin	0.78-50	$y = 31752x - 46079$	0.9940	0.128	0.427
Cinnamic Acid	0.78-50	$y = 169319x - 71126$	0.9996	0.014	0.048
Apigenin	0.78-25	$y = 484941x - 3377.5$	0.9995	0.068	0.226

Table 2. Intra-day and Inter-day Variation [% Relative Standard Deviation, (%RSD)] of the HPLC Methods

Intra-day Variation, %RSD				
Concentration ($\mu\text{g/mL}$)	Vicenin-2	Vitexin	Cinnamic Acid	Apigenin
50.0	0.88	0.42	0.57	0.40
25.0	0.74	0.58	0.79	2.12
12.50	1.69	1.17	2.42	1.57
6.25	2.17	2.17	0.74	1.12
3.12	0.89	1.88	0.81	0.76
1.56	1.00	0.86	0.12	1.06

Inter-day Variation, %RSD				
Concentration ($\mu\text{g/mL}$)	Vicenin-2	Vitexin	Cinnamic Acid	Apigenin
50.0	2.61	1.46	1.95	1.88
25.0	1.88	0.36	0.97	1.46
12.50	2.38	1.17	1.32	1.33
6.25	2.47	1.30	0.02	2.21
3.12	1.67	0.81	0.47	2.30
1.56	2.46	0.99	0.74	2.48

was used in this study for it presents the most prevalent and reliable analytical technique for analysis of polyphenolic compounds²⁸ and is the method of choice for separating complex mixtures containing non-volatile compounds such as various flavonoids in extracts prepared from different samples^{29,30}. The results of this study showed that the concentration of the four active ingredients were significantly affected by the changes in ethanolic concentration used, and 70% ethanolic extraction yielded the highest concentration of all active ingredients from Thai propolis. This finding echoes the results obtained by Sun et al. showing that the chromatogram obtained from 75% ethanolic extract of Beijing propolis had more comprehensive phenolics.¹¹ In addition, the findings are in agreement with Siripatrawan et al. such that the concentrations of their analyzed active ingredients were deemed highest at 70%.¹³ The inverse relationship between concentration of active ingredients and the concentration of ethanolic extract may be due in part to the solubility of the phenolic compounds which is dependent on the type and polarity of the solvent used, as well as the interaction to the other components of propolis samples.¹² In a study by Khacha-ananda et al., it has been shown that at 70% ethanolic extraction, the wax surrounding propolis have been effectively dissolved while its polyphenolic components and its properties are retained into the propolis extract.³¹ Using higher concentrations of ethanol during the extraction may result in excessive dissolution of not just the wax components of propolis but also its active ingredients.

Flavonoids such as vicenin-2, vitexin, and apigenin; as well as phenolic acid, specifically cinnamic acid, were the target components of our HPLC analysis. These active ingredients were chosen to be identified from the Thai propolis samples since their significance in dentistry has been proven. Vicenin-2 was documented to be effective against *E. faecalis* growth.³² A previous investigation of Thai propolis extract used as an intra-canal medicament showed a similar efficacy in inhibiting *E. faecalis* colonization.³³ On the other hand, vitexin has been documented to reduce inflammatory cytokine expression in human dental pulp stem cells.^{19,34} Apigenin and cinnamic acid have been widely studied for its biological activity against *S. mutans*, thereby preventing caries formation.^{35,36} The results of this current study corroborate the previous findings, demonstrating that flavonoids had a higher concentration, as compared to phenolic acids in Thai propolis.⁵

The anti-carries property of Thai propolis extracts and the selected active components found in Thai propolis was also investigated in this study. The findings have indicated that 70%, 80%, and 90% ethanolic extracts showed inhibitory actions against *S. mutans*, except for absolute ethanolic extract. Likewise, Thai propolis extracts exhibited activity against *L. casei*, except for the absolute ethanol extract, consistent with previous reports.^{37,38} The current results are in agreement with a previous study showing the antimicrobial effects of 20% ethanolic extract of Indian propolis in the inhibition of *S. mutans* and *Lactobacillus acidophilus* growth.³³ However,

the differences in the anti-carries potential of propolis from various sources may be due to the variation of the active components comprising the propolis extracts from other countries, and the extraction methodologies that might technically influence the harnessing of propolis extracts. The current study made use of 50 g/mL of Thai propolis that were extracted using sonication technique; whereas earlier aforementioned studies^{37,38} have used lesser amount of propolis that were extracted using maceration technique.

All four active ingredients from Thai propolis extract exhibited inhibitory effect on both *S. mutans* and *L. casei*, indicating the potential inhibition of cariogenic microbes via direct killing by vicenin-2, vitexin, apigenin, and cinnamic acid. The anti-carries potential of cinnamic acid may be through its ability to inhibit glucosyltransferase activity initiated by these bacterial species.^{23,24} Apigenin, on the other hand, may act against *S. mutans* by reducing its synthesis of extracellular glucans.²³ Moreover, a recent study demonstrated that vicenin-2, vitexin, and apigenin were also effective at inhibiting matrix metalloproteinase-13.³⁹ Having the capacity to inhibit these important mechanisms involved in the vitality of the cariogenic bacteria, these active ingredients may be able to render its anti-cariogenic potential to Thai propolis extracts. However, despite an unclear mechanism by which flavonoids and cinnamic acid utilize for the killing of *L. casei* – a secondary invader found in deep caries⁴⁰, Thai propolis extracts might still be poised for a direct inhibition of *L. casei* growth as reported earlier^{5,37,41} by the presence of four active components tested.

CONCLUSIONS

Upon analysis using HPLC, it showed that Thai propolis extracts contain vicenin-2, vitexin, apigenin, and cinnamic acid. The concentration of these active ingredients was significantly affected by the ethanol concentration used during its extraction. The study also revealed that except for the absolute ethanolic extract, all ethanolic concentrations of Thai propolis extracts, as well as its four active ingredients, have an antibacterial potential against *S. mutans* and *L. casei*, as exhibited in the agar well diffusion experiment. Further analysis on the mechanism by which the identified flavonoids and cinnamic acid utilize in the killing of these cariogenic bacteria could be looked into as future direction of the study.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

All authors declared no conflicts of interest.

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REFERENCES

- de Sousa JP, Bueno PC, Gregório LE, da Silva Filho AA, Furtado NA, de Sousa ML, et al. A reliable quantitative method for the analysis of phenolic compounds in Brazilian propolis by reverse phase high performance liquid chromatography. *J Sep Sci*. 2007 Nov;30(16):2656-65. doi: 10.1002/jssc.200700228. PMID: 17880032.
- Castro ML, do Nascimento AM, Ikegaki M, Costa-Neto CM, Alencar SM, Rosalen PL. Identification of a bioactive compound isolated from Brazilian propolis type 6. *Bioorg Med Chem*. 2009 Jul 15;17(14):5332-5. doi: 10.1016/j.bmc.2009.04.066. PMID: 19497755.
- Bruschi M, Franco S, Gremiao M. Application of an HPLC method for analysis of propolis extract. *Journal of Liquid Chromatography & Related Technologies*. 2003;26:2399-2409. doi: 10.1081/JLC-120023254.
- Boonsai P, Phuwapraisrisan P, Chanchao C. Antibacterial activity of a cardanol from Thai *Apis mellifera* propolis. *Int J Med Sci*. 2014 Feb 7;11(4):327-36. doi: 10.7150/ijms.7373. PMID: 24578609; PMCID: PMC3936026.
- Chailertvanitkul P, Namsirikul T, Damrongrungruang T, Peerapattana J. Phenolic and flavonoids contents and antibacterial activity of ethanolic extract of propolis. *Isan J Pharm Sci*. 2017;13:59-67.
- Likitpongpiat N, Sangmanceet S, Klanrit P, Noisombut R, Krisanaprakornkit S, Chailertvanitkul P. Promotion of Dental Pulp Wound Healing in New Zealand White Rabbits' Teeth by Thai Propolis Product. *J Vet Dent*. 2019 Mar;36(1):17-24. doi: 10.1177/0898756418818891. PMID: 30587093.
- Prueksakorn A, Pwasiri S, Ruangsri S, Makeudom A, Sastraruji T, Krisanaprakornkit S, et al. The preservative effect of Thai propolis extract on the viability of human periodontal ligament cells. *Dent Traumatol*. 2016 Dec;32(6):495-501. doi: 10.1111/edt.12292. PMID: 27381406.
- Bunwanna A, Damrongrungruang T, Pwasiri S, Kantrong N, Chailertvanitkul P. Preservation of the viability and gene expression of human periodontal ligament cells by Thai propolis extract. *Dent Traumatol*. 2021 Feb;37(1):123-130. doi: 10.1111/edt.12612. PMID: 33185962.
- Gómez-Caravaca AM, Gómez-Romero M, Arráez-Román D, Segura-Carretero A, Fernández-Gutiérrez A. Advances in the analysis of phenolic compounds in products derived from bees. *J Pharm Biomed Anal*. 2006 Jun 16;41(4):1220-34. doi: 10.1016/j.jpba.2006.03.002. PMID: 16621403.
- Bankova V, Trusheva B, Popova M. Propolis extraction methods: A review. *Journal of Apicultural Research*. 2021 Oct 20;60(5):734-43. Doi: 10.1080/00218839.2021.1901426
- Sun C, Wu Z, Wang Z, Zhang H. Effect of Ethanol/Water Solvents on Phenolic Profiles and Antioxidant Properties of Beijing Propolis Extracts. *Evid Based Complement Alternat Med*. 2015;2015:595393. doi: 10.1155/2015/595393. PMID: 26351514; PMCID: PMC4553316.
- Woźniak M, Mrówczyńska L, Waśkiewicz A, Rogoziński T, Ratajczak I. Phenolic profile and antioxidant activity of propolis extracts from Poland. *Natural Product Communications*. 2019 May;14(5). doi: 10.1177/1934578X19849777.
- Siripatrawan U, Vitchayakitti W, Sanguandeekul R. Antioxidant and antimicrobial properties of T hai propolis extracted using ethanol aqueous solution. *International Journal of Food Science & Technology*. 2013 Jan;48(1):22-7. doi: 10.1111/j.1365-2621.2012.03152.x
- Bueno-Silva B, Marsola A, Ikegaki M, Alencar SM, Rosalen PL. The effect of seasons on Brazilian red propolis and its botanical source: chemical composition and antibacterial activity. *Nat Prod Res*. 2017 Jun;31(11):1318-1324. doi: 10.1080/14786419.2016.1239088. PMID: 27701899.
- Ahangari Z, Naseri M, Vatandoost F. Propolis: Chemical Composition and Its Applications in Endodontics. *Iran Endod J*. 2018 Summer;13(3):285-292. doi: 10.22037/iej.v13i3.20994. PMID: 30083195; PMCID: PMC6064031.
- Šturm L, Ulrih NP. Advances in the propolis chemical composition between 2013 and 2018: A review. *Efood*. 2020 Feb;1(1):24-37. Doi: 10.2991/efood.k.191029.001
- Alanazi S, Alenzi N, Alanazi F, Tabassum H, Watson D. Chemical characterization of Saudi propolis and its antiparasitic and anticancer properties. *Sci Rep*. 2021 Mar 8;11(1):5390. doi: 10.1038/s41598-021-84717-5. PMID: 33686109; PMCID: PMC7970881.
- Righi AA, Negri G, Salatino A. Comparative chemistry of propolis from eight Brazilian localities. *Evid Based Complement Alternat Med*. 2013;2013:267878. doi: 10.1155/2013/267878. PMID: 23690840; PMCID: PMC3639640.
- Niu CG, Li JY, Yu LM, Chen D, Wei XL. Influence of vitexin on the expression of inflammatory cytokines in dental pulp stem cells induced by lipopolysaccharide. *Shanghai Kou Qiang Yi Xue*. 2020 Oct;29(5):462-465. Chinese. PMID: 33543209.
- Liaudanskas M, Kubilienė L, Žvikas V, Trumbeckaitė S. Comparison of Ethanolic and Aqueous-Polyethylenglycolic Propolis Extracts: Chemical Composition and Antioxidant Properties. *Evid Based Complement Alternat Med*. 2021 Mar 17;2021:5557667. doi: 10.1155/2021/5557667. PMID: 33815551; PMCID: PMC7994069.
- Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). *Food Chem Toxicol*. 1998 Apr;36(4):347-63. doi: 10.1016/s0278-6915(97)00145-2. PMID: 9651052.
- Gardana C, Scaglianti M, Pietta P, Simonetti P. Analysis of the polyphenolic fraction of propolis from different sources by liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal*. 2007 Nov 5;45(3):390-9. doi: 10.1016/j.jpba.2007.06.022. PMID: 17935924.
- Ikeno K, Ikeno T, Miyazawa C. Effects of propolis on dental caries in rats. *Caries Res*. 1991;25(5):347-51. doi: 10.1159/000261390. PMID: 1836157.
- Koo H, Rosalen PL, Cury JA, Park YK, Ikegaki M, Sattler A. Effect of *Apis mellifera* propolis from two Brazilian regions on caries development in desalivated rats. *Caries Res*. 1999 Sep-Oct;33(5):393-400. doi: 10.1159/000016539. PMID: 10460964.
- Choonong R, Sermpradit W, Kitisripanya T, Sritularak B, Putalun W. The contents of bibenzyl derivatives, flavonoids and a phenanthrene in selected *Dendrobium* spp. and the correlation with their antioxidant activity. *ScienceAsia*. 2019 Jun 1;45(3):245-52. Doi: 10.2306/scienceasia1513-1874.2019.45.245
- Coelho ASEC, Paula ABP, Carrilho TMP, da Silva MJRF, Botelho MFRF, Carrilho EVVF. Chlorhexidine mouthwash as an anticaries agent: A systematic review. *Quintessence Int*. 2017;48(7):585-591. doi: 10.3290/j.qi.a38353. PMID: 28555200.
- Estrela C, Estrela CR, Barbin EL, Spanó JC, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. *Braz Dent J*. 2002;13(2):113-7. doi: 10.1590/s0103-64402002000200007. PMID: 12238801.
- Luo C, Zou X, Li Y, Sun C, Jiang Y, Wu Z. Determination of flavonoids in propolis-rich functional foods by reversed phase high performance liquid chromatography with diode array detection. *Food Chemistry*. 2011;127(1):314-20. doi: 10.1016/j.foodchem.2011.01.006
- Bird IM. High performance liquid chromatography: principles and clinical applications. *BMJ*. 1989 Sep 23;299(6702):783-7. doi: 10.1136/bmj.299.6702.783. PMID: 2508923; PMCID: PMC1837629.
- Stefova M, Stafilov T, Kulevanova S. HPLC analysis of flavonoids. *Encyclopedia of chromatography*. 2003:183-95.
- Khacha-ananda S, Tragoolpua K, Chantawannakul P, Tragoolpua Y. Antioxidant and anti-cancer cell proliferation activity of propolis extracts from two extraction methods. *Asian Pac J Cancer Prev*. 2013;14(11):6991-5. doi: 10.7314/apjcp.2013.14.11.6991. PMID: 24377638.
- Filho EM, Maia CD, Bastos AC, Novais TM. Efeito antimicrobiano in vitro de diferentes medicações endodônticas e própolis sobre *Enterococcus faecalis*. *RGO*. 2008 Jan;56(1):21-5.
- Pengchum S, Chailertvanitkul P, Laopaiboon M, Thaweesit P, Abbott PV. Thai propolis extract as a root canal medication against *Enterococcus faecalis* infection. *Khon Kaen Dental Journal*. 2018; 21:30-38.

34. Kantrong N, Kumtawee J, Damrongrungruang T, Pusuiri S, Makeudom A, Krisanaprakornkit S, et al. An in vitro anti-inflammatory effect of Thai propolis in human dental pulp cells. *J Appl Oral Sci.* 2023 Jun 5;31:e20230006. doi: 10.1590/1678-7757-2023-0006. PMID: 37283330; PMCID: PMC10247283.
35. Koo H, Schobel B, Scott-Anne K, Watson G, Bowen WH, Cury JA, et al. Apigenin and tt-farnesol with fluoride effects on *S. mutans* biofilms and dental caries. *J Dent Res.* 2005 Nov;84(11):1016-20. doi: 10.1177/154405910508401109. PMID: 16246933; PMCID: PMC1490022.
36. Ribeiro M, Malheiro J, Grenho L, Fernandes MH, Simões M. Cytotoxicity and antimicrobial action of selected phytochemicals against planktonic and sessile *Streptococcus mutans*. *PeerJ.* 2018 Jun 4;6:e4872. doi: 10.7717/peerj.4872. PMID: 29888127; PMCID: PMC5991298.
37. Jafarzadeh Kashi TS, Kasra Kermanshahi R, Erfan M, Vahid Dastjerdi E, Rezaei Y, Tabatabaei FS. Evaluating the In-vitro Antibacterial Effect of Iranian Propolis on Oral Microorganisms. *Iran J Pharm Res.* 2011 Spring;10(2):363-8. PMID: 24250366; PMCID: PMC3828924.
38. Ophori EA, Eriagbonye BN, Ugbodaga P. Antimicrobial activity of propolis against *Streptococcus mutans*. *African Journal of Biotechnology.* 2010;9(31):4966-9.
39. Taherkhani A, Moradkhani S, Orangi A, Jalalvand A, Khamverdi Z. Molecular docking study of flavonoid compounds for possible matrix metalloproteinase-13 inhibition. *J Basic Clin Physiol Pharmacol.* 2020 Dec 14;32(6):1105-1119. doi: 10.1515/jbcpp-2020-0036. PMID: 34898135.
40. Ahirwar SS, Gupta MK, Snehi SK. Dental caries and lactobacillus: role and ecology in the oral cavity. *Int. J. Pharm. Sci. Res.* 2019;11: 4818-29. doi: 10.13040/IJPSR.0975-8232.10(11).4818-29
41. Martins ML, Monteiro AS, Ferreira-Filho JC, Vieira TI, Guimarães MB, Farah A, et al. Antibacterial and cytotoxic potential of a Brazilian red propolis. *Pesquisa Brasileira em Odontopediatria e Clínica Integrada.* 2019 Oct 31;19:e4626. doi:10.4034/PBOCI.2019.191.93

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