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ORIGINAL ARTICLE

The Effect of Extraction Solvents on Chemical Profile of Stingless Bee Propolis, *Trigona Apicalis*

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Abstract

Propolis is a resinous hive product known for its diverse biological activities, which are largely attributed to its complex phytochemical composition. Although the therapeutic potential of stingless bee propolis is widely acknowledged, the optimal extraction parameters for *Trigona apicalis* remain poorly characterized, particularly regarding how solvent polarity influences the yield and efficacy of its bioactive constituents. Therefore, the present study aims to investigate the influence of different extraction solvents on the chemical profile of stingless bee propolis, *T. apicalis*. Propolis samples were subjected to maceration for 72 hours using different polarity solvents such as hexane, ethyl acetate and methanol. The resulting extracts were characterized via Ultraviolet (UV)-Visible and Fourier-transformed infrared (FTIR) spectroscopy. UV-Vis analysis revealed distinct chromophore patterns across the 200–500 nm range. The aromatic chemicals are represented by peaks at range 260–280 nm whereas flavonoids and phenolic structures are identified by peaks at the region 300–380 nm. Additionally, FTIR spectroscopy demonstrated a comprehensive fingerprint of functional groups including O-H stretching, C=O stretching vibrations and C-O esters or phenol. These spectroscopic data suggest differences in the distribution of polyphenolics and other bioactive constituents among the solvent extracts, reflecting the importance of solvent selection in optimizing the extraction of bioactive constituents.

Keywords: Extraction solvent; FTIR spectroscopy; UV-Vis spectroscopy; *Trigona apicalis*.

Introduction

There are approximately 500 species within the stingless bee genus, with most of these species being in Latin America, the mainland of Australia, Africa and Eastern and Southern Asia (Garibaldi et al., 2017). Stingless bees (tribe Meliponini) exhibit remarkably high species diversity, far exceeding the limited number of species within the honeybee genus *Apis mellifera linnaeus* (Abd

Jalil et al., 2017). Stingless bees also play an important role in the ecology, economy and culture. They act as the main pollinators for many wild and cultivated tropical plants (Rocha et al., 2023).

Their products such as honey, propolis and pollen have been used as a source of income for human generations. Propolis is a complex resinous substance manufactured by stingless bees and these resins are used to line the inside of nest cavities and reduce the size of hive entrances (Ibrahim et al., 2018). The composition of propolis depends on the type of plants accessible to the bees. Propolis has a wide range of biological activities such as antibacterial, anti-inflammatory, antiulcer, antioxidant, hepatoprotective, tumoricidal activities and high repellent index against ants (Salleh et al., 2021). It is generally accepted that bees collect resinous plant materials produced by a variety of botanical processes in different parts of plants to produce propolis (Bankova et al., 2018).

Propolis consists of a wide range of chemical constituents depending on geographical origins, bee species, botanical sources and harvesting season (Tagliacollo & Orsi 2011; Huang et al., 2014). A study by Salleh et al. (2021) reported that propolis from three stingless bee species from Malaysia were primarily composed of sugars (31.4%), followed by carboxylic acids (17.1%), terpenoids (14.3%) and sugar alcohols (11.4%). Minor components identified included hydrocarbons and aldehydes (5.7% each), amino acids (2.9%) and various other constituents (11.4%). Other compounds that have been identified including 1H-Pyrrole-2-carboxylic acid,1-(2-hydroxy-2-phenylethyl), fren-9(11)-en-2.alpha.ol, limonoids, hexamethylcyclotrisiloxane, 2-hydroxy-2-cyclopenten-1-one and 2-coumaranone in propolis from Malaysia (Nazir et al., 2018; Mahmad et al., 2025; Zohdi et al., 2023).

The *Trigona apicalis* propolis contains significant amounts of terpenes, including triterpenes such as amyrin types, which are typical of dipterocarp trees (Popova et al., 2022). This indicates that Malaysian stingless bee propolis is rich in diverse bioactive compounds, including phenolics, flavonoids, terpenoids and various other chemical groups. These compounds contribute to the propolis's therapeutic potential, particularly its antioxidant and antibacterial properties.

Nowadays, propolis produced by stingless bees such as *T. apicalis* has gained increasing attention due to its potential therapeutic and functional properties. However, the chemical profile of propolis is highly influenced by the extraction method, especially the type of solvent used. Different solvents vary in polarity and extraction efficiency, which can lead to significant differences in the types and concentrations of bioactive compounds obtained. A study by Mello et al. (2010) reported that propolis extracted with ethanol produce more polar compounds like phenolic acids, while water extracts yield fewer bioactive compounds.

Aqueous extraction demonstrates lower efficacy compared to ethanolic extraction due to the limited solubility of various phenolic and flavonoid compounds in water (Peixoto et al., 2025; Yurt, 2023; Rasgele et al., 2023). This lack of standardized extraction data hinders accurate comparison between studies and limits the optimal utilization of propolis in pharmaceutical, nutraceutical and food applications. Therefore, this study aims to investigate the effect of different extraction solvents on the chemical profile of stingless bee propolis *T. apicalis* using UV-Vis analysis and FTIR spectroscopy. The findings will support method optimization, improve interpretation of spectroscopic data and contribute to standardized analytical approaches for propolis characterization.

Materials and Methods

Preparation of Propolis Extract

Propolis *T. apicalis* was collected at Masjid Al-Sultan Abdullah Ri'Ayatuddin Al-Mustafa Billah Shah, Jengka, Pahang Malaysia (coordinate: 3.766051503848031, 102.54504772698192) and

stored in the chiller. Through this procedure, hexane, ethyl acetate and methanol were selected to represent non-polar, moderately polar and polar solvents, respectively. Approximately 30 g of propolis was weighed and macerated with 70 mL of different solvents for 72 hours.

The process was conducted at room temperature under static conditions with occasional manual stirring, completed as a single extraction cycle. Each solvent extraction was conducted in a separate conical flask. The propolis was evaporated using rotatory evaporation at 40° C to remove the solvents. Then the samples were kept in the chiller prior to analysis.

Ultraviolet-visible Spectroscopy (UV-Vis) Analysis

The UV-visible spectral data were recorded using a spectrophotometer equipped with a deuterium lamp for the UV range and a tungsten-iodine lamp for the visible range. Quartz cuvettes with a 10 mm path length were used for both sample and blank measurements, with ethanol serving as the blank solvent.

Spectral scanning was conducted from 200 to 500 nm at 1 nm intervals, with a fixed measurement speed of 400 nm/min. Baseline autocorrection was applied to all samples before analysis.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

ATR-FTIR spectra were obtained using the IRPrestige-21 Shimadzu Fourier Transform Infrared (FTIR) spectrophotometer (Tokyo, Japan) equipped with an air-cooled ceramic infrared light source and a DLATGS (Deuterated Triglycine Sulfate doped with L-Alanine) detector. The spectral range was set from 4000 cm^{-1} to 500 cm^{-1}.

Samples were analyzed directly on a diamond prism. The resolution for IR measurements was 4 cm^{-1}, and 16 interferograms were co-added before Fourier transformation. FTIR data processing, including baseline correction, normalization and smoothing was carried out using Shimadzu IRsolution software (version 1.40). Baseline autocorrection was also performed before each measurement.

Results and Discussion

Chemical Profile of Propolis Using UV-Vis Analysis

UV-Vis reflectance spectroscopy has been widely used as a non-invasive method for the study of cultural heritage materials for several decades (Picollo et al, 2019). UV-Vis spectroscopy revealed a distinct profile of propolis, a resinous material made by bees. The preliminary chemical profile will provide an insight into chemical components such as flavonoids, phenolic acids and their derivatives that present in the propolis. Propolis usually exhibits distinctive absorption bands in the region of 200-500 nm.

The aromatic chemicals are represented by peaks at range 260-280 nm whereas flavonoids and phenolic structures are identified by peaks at the region 300-380 nm. Differences in propolis' botanical source, regional origin and extraction techniques may cause variations in the spectrum. Because of these unique spectral characteristics, UV-Vis is a useful instrument for assessing the quality and chemical structure of propolis (Fig. 1).

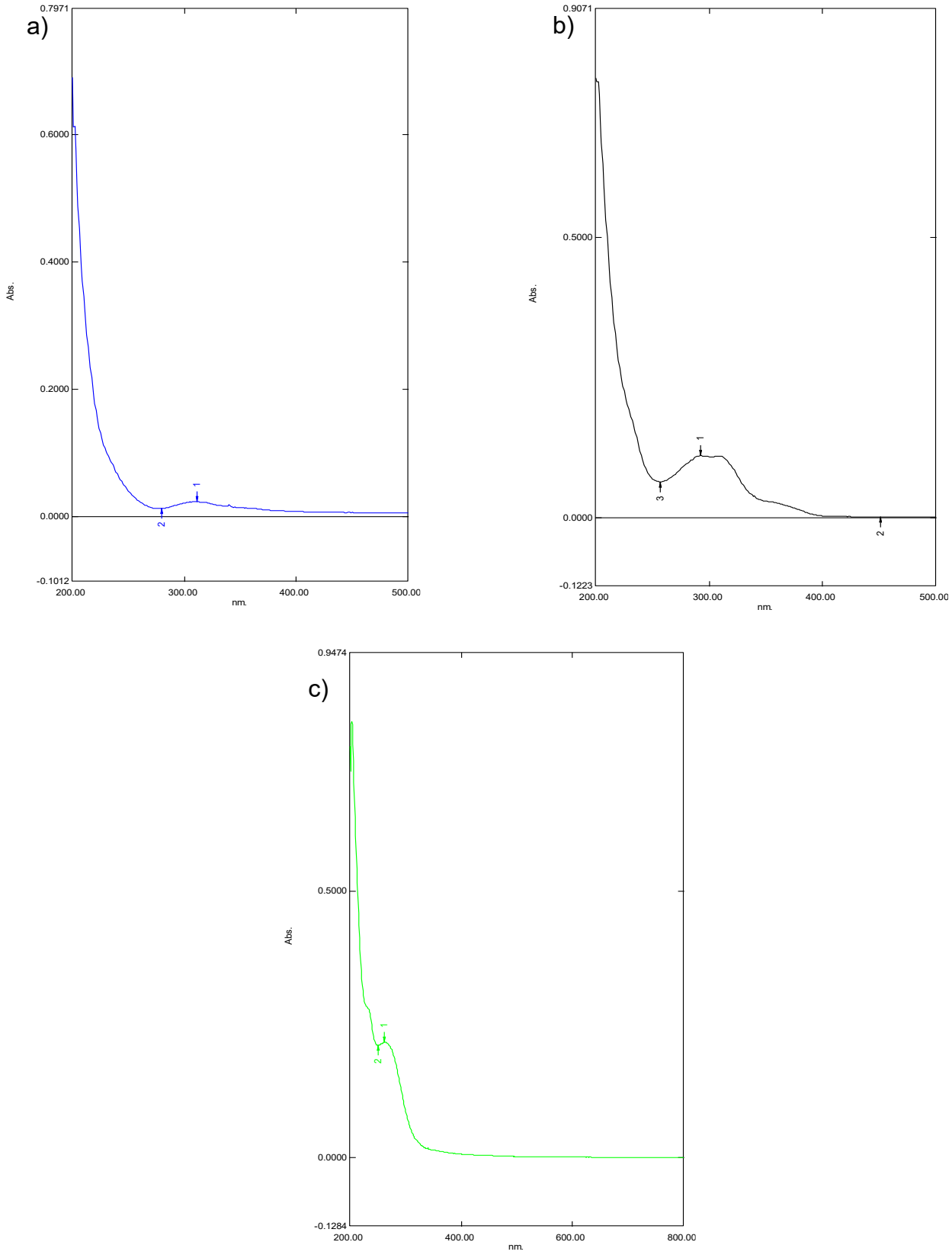


Figure 1. The UV-Vis spectra of propolis from stingless bee *T. apicalis* from different types of solvent (a) hexane, (b) ethyl acetate and (c) methanol

As shown in Fig. 1, all UV-Vis spectra propolis from different types of solvent revealed distinct absorption peaks which correspond to the functional groups and bioactive compounds present in the propolis extract. These spectral variations are primarily governed by the differential solubility of polar and non-polar phytochemicals according to the polarity index of the extraction solvents used. Based on Fig. 1 (a), the UV-Vis spectra hexane extract displayed a prominent absorption peak at 280 nm which corresponds to the presence of flavonoids and phenolic compounds commonly found in propolis (Lesmana et al., 2024). This peak is characteristic of the aromatic ring structure of flavonoid compounds. Additionally, a secondary peak was observed at 311 nm, which could indicate the presence of terpenoids or other hydrophobic compounds soluble in hexane. Typically, this involves $\sigma \rightarrow \sigma^*$ transitions for saturated hydrocarbons, but $\pi \rightarrow \pi^*$ transitions could occur with unsaturation. The intensity of the peak at 311 nm suggests a moderate concentration of the active compounds in the hexane extract.

Furthermore, the UV-Vis spectra of ethyl acetate extract as observed Fig. 1 (b) showed a distinct peak at 271 and 292 nm consistent with the absorption of flavonoids and phenolic acids. These peaks possible indicate the presence of aromatic rings or conjugated phenolics or ester compounds that are soluble in ethyl acetate. This could be due to $n \rightarrow \pi^*$ transitions from the carbonyl group or $\pi \rightarrow \pi^*$ if there's conjugation. This suggests that ethyl acetate extract is moderately effective in extracting bioactive compounds from propolis. In line with Tomazzoli et al. (2015), the presence of phenolic compounds was confirmed through UV-Vis profiling, specifically within the region 280 and 400 nm. From Fig. 1 (c), the UV-Vis spectra of methanol extract exhibited a stronger peak at 262 nm which is typically associated with phenolic compounds and flavonoids that are more soluble in polar solvents such as methanol (Dong et al., 2023). This transition might be $\pi \rightarrow \pi^*$ if there's conjugation or $n \rightarrow \pi^*$ due to the lone pair on oxygen. To conclude, the UV-Vis spectroscopy provided a clear distinction between the extracts, offering insights into their potential applications. Future studies might investigate the stability of these extracts or their specific actions against various biological targets.

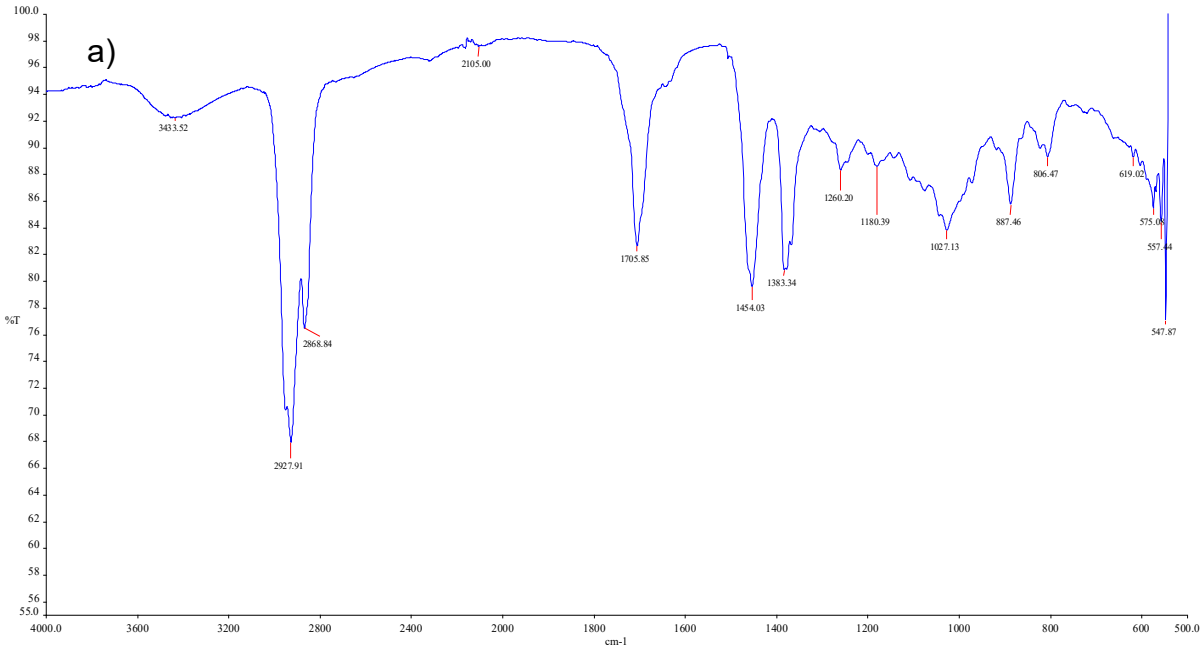
Chemical Profile of Propolis Using FTIR Analysis

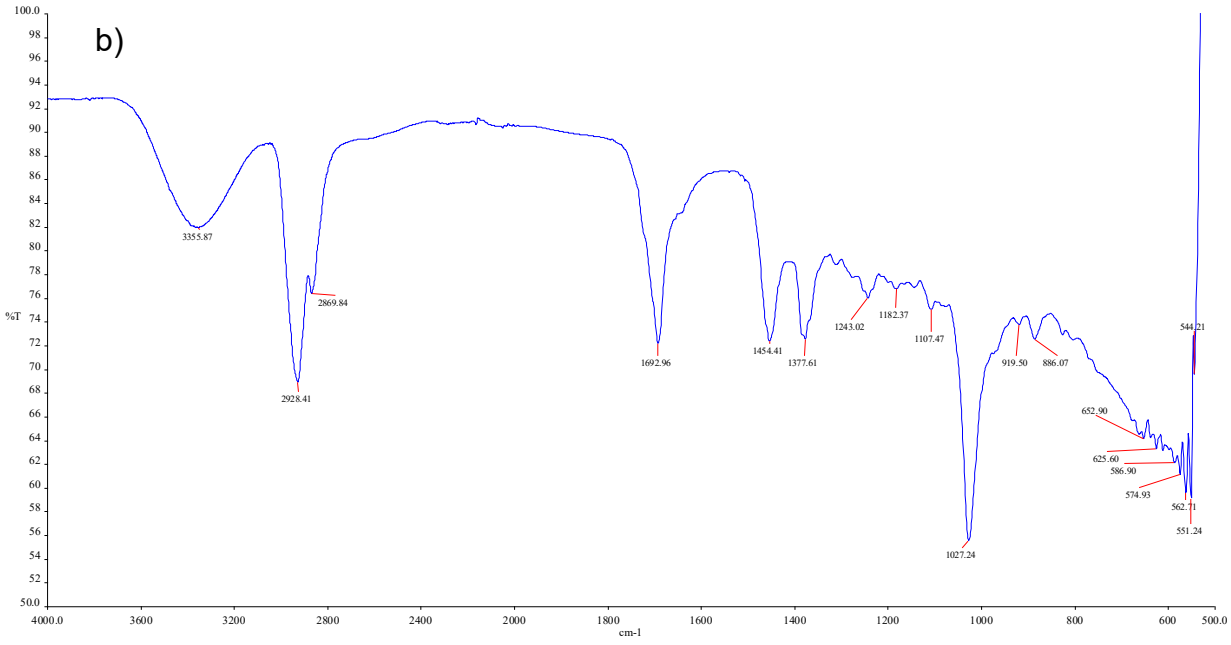
FTIR spectroscopy was employed to determine the functional groups in the propolis, revealing chemical bonds that reflect the major bioactive components in the *T. apicalis* propolis. Fig. 2 illustrates the vibrational spectra of *T. apicalis* propolis extracts obtained using three different solvents: (a) hexane, (b) ethyl acetate and (c) methanol. The observed spectral variations reflect differences in the chemical composition of each propolis extract. The vibrational bands and their corresponding functional group are summarized in Table 1. A uniform spectral fingerprint was observed among the samples, however there were differences in signal intensity, reflecting the differing solvating powers of the solvents used.

The FTIR spectra in Fig. 2 revealed a broad band between 3500 to 3200 cm^{-1} in all extracts with different signal intensities observed. This characteristic peak is attributed to O-H stretching vibrations typically associated with hydroxyl groups in alcohols or phenolic compounds (Dai et al., 2023). Two bands with strong intensities observed in all propolis extracts (hexane: 2927, 2868, ethyl acetate: 2928, 2899 and methanol: 2928, 2869 cm^{-1}) corresponded to CH_3 and CH_2 stretching vibrations. These spectral features were attributed to the presence of aliphatic hydrocarbons. The strong peak at 1708 cm^{-1} indicated the presence of C=O ketone aliphatic stretching in hexane and methanol extracts. A strong, sharp peak at 1692 cm^{-1} showed the presence of C=O conjugated acid stretching which correlate with flavonoids or lipids.

This peak was detected only in ethyl acetate extract. Special features of phenolic groups were also observed for interaction of C-O esters or phenol (1238 cm^{-1}) and C-O primary alcohol (1045-1027 cm^{-1}). The detected polyphenol signals and features are consistent with previous study by Ibrahim et al. (2018) and Zin et al. (2019) in Malaysian stingless bee species, *G. thoracica* and *H. itama* propolis. Furthermore, these results corroborate with the FTIR signals reported by

Badiazaman et al. (2023), suggesting the presence of flavonoids, phenolics, terpenoids, and sterols in *G. thoracica* propolis. Moreover, these findings are supported by existing literature reporting the occurrence of polyphenolic constituents within propolis samples based on the signals above (Masek et al., 2019; Akbar et al., 2022; Nisa et al., 2022).





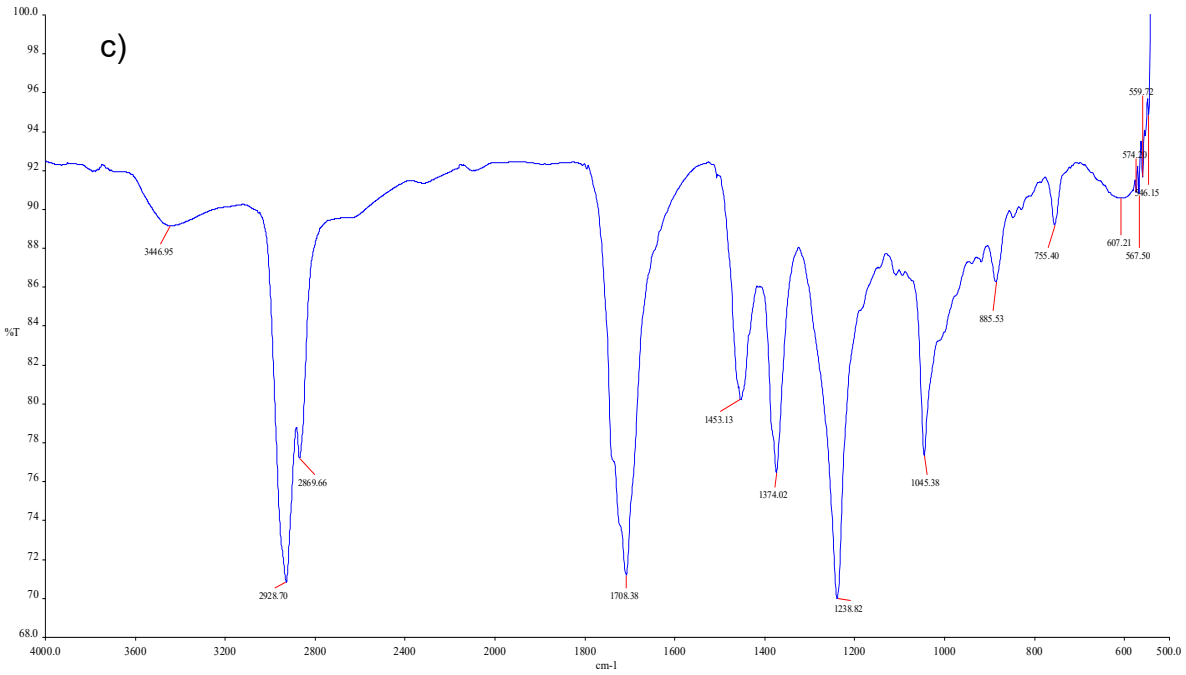


Figure 2. The FTIR spectra of propolis from stingless bee *T. apicalis* from different types of solvent (a) hexane, (b) ethyl acetate and (c) methanol.

Table 1. FTIR peak assignments for *T. apicalis* propolis from different extraction solvents.

Wavenumbers (cm^{-1})	Functional Groups	Hexane	Ethyl acetate	Methanol
3446-3355	OH stretching	Present	Present	Present
2928-2927	CH_3 stretching	Present	Present	Present
2899-2869	CH_2 stretching	Present	Present	Present
1708	C=O ketone aliphatic	Present	Absent	Present
1692	C=O conjugated acid	Absent	Present	Absent
1454	CH_3 and CH_2 bending	Present	Present	Present
1383-1374	CH_3 symmetrical bending	Present	Present	Present
1238	C-O esters or phenol	Absent	Absent	Present
1045-1027	C-O primary alcohol	Present	Present	Present
887-885	C-H out of plane bending	Present	Present	Present

Conclusion

The comparison chemical profiles of propolis from different extraction solvents analysed via UV-Vis and FTIR spectroscopy revealed clear distinctions among the extracts. UV-Vis spectroscopic analysis revealed distinct spectral patterns for each extract, with characteristic absorption peaks in the 200–500 nm range. These peaks correspond to the specific electronic transitions of phenolic and flavonoid chromophores, indicating variation in the concentration of these bioactive constituents across the different solvents.

Additionally, FTIR spectroscopy revealed comparable spectral profiles across all extracts, suggesting a fundamental similarity in their qualitative chemical composition and functional group distribution such as O-H stretching, C=O stretching vibrations and C-O esters or phenol. These findings highlight the importance of solvent used to extract the bioactive compounds. Future investigations should utilize HPLC analysis to quantify key bioactive markers, facilitating the development of a standardized chemical fingerprint for *T. apicalis*.

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