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ESTUDO QUÍMICO E POTENCIAL BIOLÓGICO DE MEL E PRÓPOLIS DE *Scaptotrigona depilis*

EDINEIDE CRISTINA ALEXANDRE DE SOUZA

Boa Vista-RR

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Tese de doutorado apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Biodiversidade e Biotecnologia-Rede BIONORTE, na Universidade Federal de Roraima, como requisito parcial para a obtenção do Título de Doutor em Biodiversidade e Biotecnologia.

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Co-orientadores: Dr. Cristiano Menezes

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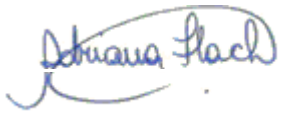

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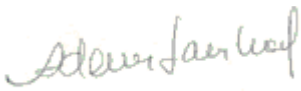

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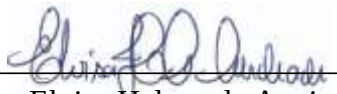

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RESUMO

Os produtos elaborados por abelhas sempre despertaram o interesse, seja pelos benefícios terapêuticos ou ainda pelo sabor agradável do mel como aditivo culinário. Com o passar dos anos a aplicação desses produtos ficou mais ampla, em cosméticos, fármacos e nutricionais, cujas propriedades foram mais bem definidas. A caracterização química é parte fundamental para o conhecimento dos benefícios e utilidades desses. Em virtude do exposto, este estudo tem como objetivo determinar a composição química do mel submetido a diferentes tratamentos, e da própolis produzidas por *Scaptotrigona depilis*. Para o estudo da composição de própolis, foram coletadas amostras em colônias de *S. depilis*, criadas no meiponário da Embrapa Meio Ambiente, em Jaguariúna-SP, das quais foram elaborados extratos com álcool etílico 70% (EPE70) e álcool de cereais (CAPE). Os extratos foram analisados por LC-ESI-MS/MS. A própolis *in natura* também foi submetida à extração de voláteis por hidrodestilação que foram analisados por cromatografia a gás acoplada a espectrometria de massas (CG-EM). Além disso, foram realizados ensaios para determinação da atividade antimicrobiana frente a patógenos de interesse clínico. Em relação ao mel, foram realizados tratamentos: pasteurização, desumidificação, refrigeração e maturação por 180 dias. O mel fresco assim como os méis tratados foram extraídos voláteis dos méis por meio da técnica de headspace no modo dinâmico, utilizando Porapak-Q com fluxo de gás nitrogênio, os compostos extraídos foram caracterizados por CG-EM. Foram quantificados fenólicos pelo método de Folin-Ciocalteu e flavonoides com cloreto de alumínio, além disso, foi determinada a atividade antioxidante pelo método do sequestro do radical livre DPPH dos méis tratados e *in natura*. Foram ainda realizados teste de análise sensorial, por meio da análise de ordenação-preferência e aceitação empregando a escada hedônica, com provadores não treinados. Os constituintes voláteis presentes na própolis são pertencentes à classe de sesquiterpenos. Nos extratos da própolis os perfis cromatográficos evidenciaram que o extrato EPE70 apresenta maior número de sinais. Após análises de dados em software apropriado, por meio da análise de conjunto usando o diagrama de Venn, observou-se maior quantitativo de entidades químicas exclusivas no EPE70. Através do Molecular Networking (MN) foi possível sugerir a presença de diferentes classes de compostos, em que os mais representativos foram os terpenos com erros inferiores a 5 ppm. Os extratos etanólicos da própolis de *Scaptotrigona depilis* apresentaram atividade antimicrobiana frente a *Echerichia coli* e *Staphylococcus aureus*. As análises dos voláteis dos méis sugerem perfis diferenciados após tratamento ao longo de 180 dias. O teor de fenólicos não sofreu alterações significativas ao longo do período de análise, e consequentemente sua atividade antioxidante também não sofreu. A análise sensorial evidenciou que apenas os atributos cor, sabor e aparência global apresentaram diferenças significativas entre o mel maturado e o in natura e, apesar disso a maior preferência é pelo mel in natura. O estudo é uma importante contribuição às pesquisas com produtos elaborados por esta espécie de abelha e poderá subsidiar a regulamentação e padronização do beneficiamento do mel de abelhas sem ferrão.

Palavras-cheve: Composição química, perfil sensorial, voláteis, *Molecular Networking*.

SOUZA, Edineide Cristina Alexandre de. **Chemical study and biological potential of *Scaptotrigona depilis* honeys and propolis**. 2022. 104f. Thesis (PhD in Biodiversity and Conservation) - Federal University of Roraima, Roraima, RR-Brazil, 2022.

ABSTRACT

Products made by bees have always aroused interest, either for the therapeutic benefits or for the pleasant taste of honey as a culinary additive. Over the years, the application of these products became wider, in cosmetics, pharmaceuticals and nutritionals, whose properties were better defined. Chemical characterization is a fundamental part of understanding their benefits and uses. In view of the above, this study aims to determine the chemical composition of honey subjected to different treatments, and of the propolis produced by *Scaptotrigona depilis*. For the study of the composition of propolis, samples were collected in colonies of *S. depilis*, created in the meiponário of Embrapa Meio Ambiente, in Jaguariúna-SP, from which extracts were prepared with 70% ethyl alcohol (EEP70) and cereal alcohol (EPAC). Extracts were analyzed by LC-ESI-MS/MS. In natura propolis was also subjected to the extraction of volatiles by hydrodistillation, which were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). In addition, assays were performed to determine the antimicrobial activity against pathogens of clinical interest. Regarding honey, treatments were carried out: pasteurization, dehumidification, refrigeration and maturation for 180 days. Fresh honey as well as treated honeys were extracted from honey volatiles by headspace technique in dynamic mode, using Porapak-Q with nitrogen gas flow, the extracted compounds were characterized by GC-MS. Phenolics were quantified by the Folin-Ciocalteu method and flavonoids with aluminum chloride, in addition, the antioxidant activity was determined by the DPPH free radical scavenging method of the treated and in natura honeys. Sensory analysis tests were also carried out, through the analysis of ordering-preference and acceptance using the hedonic ladder, with untrained tasters. The volatile constituents present in propolis belong to the class of sesquiterpenes. In the propolis extracts, the chromatographic profiles showed that the EEP70 extract presents a greater number of signals. After data analysis in appropriate software, through ensemble analysis using the Venn diagram, a greater quantity of unique chemical entities was observed in EEP70. Through Molecular Networking (MN) it was possible to suggest the presence of different classes of compounds, in which the most representative were terpenes with errors below 5 ppm. The ethanolic extracts of Scaptotrigona depilis propolis showed antimicrobial activity against *Echerichia coli* and *Staphylococcus aureus*. The analyzes of honey volatiles suggest different profiles after treatment over 180 days. The phenolic content did not change significantly over the period of analysis, and consequently its antioxidant activity did not change either. The sensory analysis showed that only the attributes color, flavor and overall appearance showed significant differences between matured and in natura honey and, despite this, the greatest preference is for in natura honey. The study is an important contribution to research with products made by this species of bee and may support the regulation and standardization of the processing of honey from stingless bees.

Keywords: Chemical composition, sensory profile, volatiles, Molecular Networking.

SUMÁRIO

1. INTRODUÇÃO

As abelhas contribuem substancialmente para a diversidade da Amazônia, por meio do serviço de polinização, inúmeras plantas podem se reproduzir, proporcionando a produção de alimentos, além da manutenção e conservação de relações ecológicas entre plantas e animais. A diversidade vegetal aliada a diversidade de espécies de abelhas presentes na Amazônia impulsionam pesquisas nos âmbitos ecológico, químico e biológico. Os produtos elaborados pelas abelhas apresentam uso milenar, em que suas propriedades ao longo do tempo foram sendo elucidadas.

O mel produzido no Brasil é predominantemente da abelha *Apis mellifera*, uma espécie introduzida que possui grande potencial produtivo. Entretanto, a criação de abelhas desta espécie é marcada pela dificuldade no manejo das colmeias em virtude da defensividade destes insetos. Nesse contexto, surgem as abelhas-sem-ferrão, cujas defesas são menos danosas e, apesar da produção de mel ser menor, possuem características peculiares que influenciam a diversidade química e, consequentemente, o potencial biológico.

A composição química dos méis e outros produtos das abelhas, como própolis e pólen é bastante variada, tendo em vista a diversidade de plantas e espécies de abelhas presente no país. Determinar os constituintes que compõem a própolis é algo bem mais consolidado, já que existem padronizações que regulam a obtenção de extratos para comercialização. Já para o mel, os trabalhos envolvendo a caracterização de compostos secundários ainda são poucos, o que pode ser justificado pela complexidade desta matriz, por ser uma solução saturada de açúcares a obtenção de extratos pode gerar artefatos que dificultam a análise.

Estudos apontam diferenças nos parâmetros físico-químicos dos méis de abelhas-sem-ferrão, onde a principal citada é o teor de umidade e a acidez, o tem como consquencia a fermentação do mel. Isso porque, as estratégias para estoque do mel por parte das abelhas se diferem entre *Apis mellifera* e abelhas-sem-ferrão. No caso das *Apis mellifera* as abelhas retiram a umidade até um determinado nível em que os microrganismos não conseguem mais se reproduzir e com isso pode ficar estocado por muitos anos sem deteriorar e mantendo praticamente as mesmas características de cor, sabor, aroma e propriedades físico-químicas. Já a estratégia usada pelas abelhas sem ferrão é diferente, elas desidratam o mel razoavelmente, porém até um nível específico (geralmente em torno de 75%), no qual os microrganismos ainda conseguem se reproduzir e utilizá-lo. Devido a esta menor desumidificação os méis de grande parte das abelhas nativas tende a fermentar e alterar seu aroma, cor e sabor, sendo muitas vezes rejeitado pelos consumidores. Uma maneira de

diminuir a fermentação é aplicar tratamentos que diminuam esse processo e que aumentem a aceitabilidade do mel. A análise sensorial é uma etapa importante do estudo, tem como objetivo conhecer as variáveis de produto de acordo com a percepção do público, em que são discriminados os atributos do mel, permitindo a identificação e avaliação da intensidade dos atributos sensoriais (ARNAUD et. al., 2008).

Nosso grupo de pesquisa tem procurado estudar as propriedades alimentícias e também os compostos secundários e atividades biológicas de abelhas nativas e de *Apis mellifera*. Para este estudo foram selecionados os tratamentos: pasteurização, refrigeração, desumidificação e maturação, já descritos na literatura como formas de beneficiamento do mel de abelhas sem ferrão.

Diante do exposto, este trabalho tem como objetivo determinar o perfil químico do mel e própolis produzidos por *Scaptotrigona depilis*, bem como avaliar atividade biológica desses produtos, além da análise dos parâmetros físico-químicos do mel submetido a processos de conservação e perfil sensorial. A pesquisa está organizada em capítulos que tratam individualmente de cada produto, buscando atender os objetivos propostos. O Capitulo 1 é uma revisão de literatura que apresenta o estado da arte da pesquisa com mel de abelhas sem ferrão, discutindo os resultados obtidos e apresentando as perspectivas futuras para impulsionar o estudo desse produto. O Capitulo 2 versa sobre a composição volátil do mel submetido a diferentes tratamentos, o teor de fenólicos e atividade antioxidante determinados após 180 dias de estocagem dos méis tratados, além dos parâmetros físico-químicos e perfil sensorial. O capítulo 3 é uma breve revisão bibliográfica sobre própolis de abelhas sem ferrão, apresentando os dados sobre sua composição química e seu potencial biológico das mesmas. No capítulo 4 estão compilados os dados de caracterização química da própolis por UHPLC-MS/MS guiada pela técnica de análise de agrupamentos de espectro, o *Molecular Networking*. A composição de voláteis e quantificação de compostos fenólicos, bem como a atividade antimicrobiana também são descritas.

Desta forma insta destacar que os resultados obtidos darão suporte à normas para o controle de qualidade do mel de abelhas-sem-ferrão. Apesar da diversidade e a peculiaridade de cada uma delas, considera-se relevante a sua aplicação, já que ainda são poucos os estudos com esse direcionamento. Além disso, o modelo experimental que tem a espécie *Scaptotrigona depilis*, de vasta distribuição, bem como o ambiente controlado em que elas são criadas proporciona quantidades suficientes de mel para o estudo proporcionando resultados que subvencionarão pesquisas futuras.

1.2 OBJETIVOS

Geral:

Determinar o perfil químico do mel e própolis produzidos por *Scaptotrigona depilis*, bem como avaliar atividade biológica desses produtos, além da análise dos parâmetros físico-químicos do mel submetido a processos de conservação e perfil sensorial.

Específicos:

- Investigar a composição química da própolis de *Scaptotrigona depilis* a partir de extratos etanolólicos caracterizados por UHPLC-ESI-MS/MS empregando ferramentas de desreplicação da plataforma GNPS (*Global Natural Products Social Molecular Networking*);
- Avaliar o potencial biológico por meio da atividade antimicrobiana dos extratos etanólicos da própolis;
- Investigar a composição química do mel de *Scaptotrigona depilis* submetido a diferentes tratamentos a partir da caracterização de compostos voláteis obtidos pelo método headaspace em modo dinâmico;
- Determinar o teor de fenólicos, potencial antioxidante e parâmetros de qualidade de amostras de méis de *Scaptotrigona depilis* submetido a diferentes tratamentos;
- Investigar a composição dos méis a partir da caracterização de compostos voláteis obtidos pelo método headaspace em modo dinâmico;
- Avaliar o perfil sensorial por meio da análise de ordenação-preferencia dos méis submetidos aos tratamentos.

Capítulo 1

Stingless bees honey (Hymenoptera, Apidae): A review of quality control, chemical profile and biological potential.

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Review article

Stingless bee honey (Hymenoptera, Apidae, Meliponini): a review of quality control, chemical profile, and biological potential

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Abstract – Products made by bees are well-known for their beneficial properties and nutritional value. This association has been proven by scientific studies that describe their composition and biological activities. The aim of this study is to portray the state of the art on research regarding stingless bee honey. The search for standards that guide the trade of these products is still portrayed as a future perspective, since there are significant differences in relation to honey from *Apis mellifera* and it often requires additional treatments.

stingless bees / honey quality / chemical composition

1. INTRODUCTION

Stingless bees, also called meliponines, native, or indigenous bees, comprise a wide group of eusocial bees and present a range of variations in behavioral aspects, communication systems, foraging strategies, population densities, and nest architectures, among others (Nogueira-Neto 1997). More than 500 species of stingless bees have been described and 61 genera are distributed in Latin America, Australia, Africa, and tropical parts of Asia.

The use of rational breeding techniques, knowledge of the explored flora, the implementation of management techniques, and artificial feeding have allowed the expansion of meliponiculture (Jaffé et al. 2015). Many species are popular and are raised to obtain products which generate jobs and income and, at the same time, maintain biodiversity (Kerr et al. 2001; Imperatriz-Fonseca and Nunes-Silva 2010; Contrera et al. 2011; Ollerton et al. 2011; Freitas and Nunes-Silva 2012; Bartelli and Nogueira-Ferreira 2014). In Brazil, many tree species and agricultural crops are pollinated by these bees and their effective pollination performance has been confirmed for more than 30 different agricultural crops (Heard 1999; Slaa et al. 2006; Castro et al. 2006). The importance of *Apis mellifera* in pollination has already been widely reported (Blettler et al. 2018) whereas for stingless bees, studies have been conducted to evaluate pollination efficiency considering the productive increase of cultivars, and the results indicate that these bees are considered promising for use as commercial pollinators (Roselino et al. 2009, 2010; Kiatoko et al. 2014).

In the Amazon region, there is a great diversity of bees, which can be attributed to the favorable

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conditions, such as warm weather and flora rich in species that supply nectar, pollen, and resins. In these regions, the honey has been the main product of extraction; however, studies have shown that many species of meliponines have considerable productive potential for propolis, geopropolis, batumens, and cerumens, in addition to pollen (Contrera et al. 2011).

In the last decade, researchers and environmentalists from around the world have shown great concern about the decline in the population of managed and wild pollinators (Potts et al. 2016), and have promoted a significant scientific advance related to the theme of bees. Many campaigns are being carried out to publicize their importance to human existence and in the maintenance of the ecosystem, and thus provoke a wave of worldwide interest on the subject and open new horizons for scientific research, especially regarding stingless bees, which are still little studied. Some recent reviews have been published on topics such as propolis (Anjum et al. 2018; Popova et al. 2019), reproductive behavior (Vollet-Neto et al. 2018), and palynological analysis (Souza et al. 2019). This review aims to discuss what the scientific community knows about honey from stingless bee honeys, when used not only as food but also its functional properties.

2. HONEY

Most studies regarding honey have been carried out with *Apis mellifera* , since this species has adapted to different regions around the world. However, the literature extolls the virtues of the different characteristics of honey from stingless bees, especially in relation to its moisture content, peculiar flavor, and more pronounced aroma (Alves et al. 2005).

The honey production strategy of *A. mellifera* consists of removing the moisture from the nectar to a certain level that the microorganisms can no longer reproduce and with that it can be stored for many years without deteriorating and maintaining practically the same characteristics of color, flavor, aroma, and physicochemical properties. Bees remove moisture by using their wings and add enzymes with the function of digesting sugars and conserving honey. Honey is stored in combs made of wax produced by the workers' glands and after being closed, the honey no longer has contact with air (Seeley 1985).

The strategy used by stingless bees is different. They dehydrate honey to a reasonable, however, specific level (Vit et al. 1994; Souza et al. 2006). After being stored, microorganisms, mainly bacteria of the genus *Bacillus* and yeasts, will consume part of the sugar and transform it into alcohol through anaerobic fermentation and then this alcohol is transformed into acetic acid through aerobic fermentation. Sugar can also be transformed into other types of acids (and other by-products) through other types of non-alcoholic fermentation (Gilliam et al. 1985; Gilliam et al. 1990; Menezes et al. 2013). This fermentation alters the characteristics of the honey and is very specific, since each bee species has its own microbiome and the processing dynamics are different. There is evidence that microorganisms also add enzymes and other compounds to honey that can contribute to its conservation and digestion of nutrients (Menezes et al. 2013). In addition, the cerumen pot, in contrast to the *Apis mellifera* honeycomb, gradually transfers the aromas from the pots to the honey and, via their intensity, superimposes them on the honey, thus creating the specific identity of the bee species. It is possible that bioactive substances, such as antibiotics and antioxidants, are being incorporated into honey in these processes.

The composition of honey depends, among other factors, on the plant sources from which it is derived, the species of the bee, the physiological state of the colony, the state of maturity of the honey, and the weather conditions during the harvest (Campos et al. 2003). The honey of the meliponines species has as its main characteristic, the highest acidity and the highest water content (moisture), which makes it less dense than the honey from Africanized bees (*A. mellifera*). The chemical composition has been little studied, and the studies that exist are limited to the quantitative determination of its phenolic and flavonoid compounds.

Due to the particularities presented by honey from native bees, studies aiming at characterization have been carried out, with the objective of

determining their identity and controlling possible adulterations. These studies are important for the elaboration of a legislation that meets the quality control of honey of meliponines (Vit et al. 1994; Souza et al. 2006). In Brazil, the legislation regarding honey is intended for the classification of honey from *A. mellifera* (Brazil 2000) and does not deal with the characteristics of the product of stingless bees. Recently, however, some Brazilian states such as Bahia (Brazil 2014) and São Paulo (Brazil 2017) have defined specific parameters for honeys from stingless bees, aiming at quality control and the formalization of the sale of this product. With this, establishments that aim at the processing of honey and are accredited by the Federal Inspection Service are already managing to overcome the pre-existing bureaucratic barriers, register the honey from stingless bees, and commercialize it in the formal market.

3. PHYSICO-CHEMICAL PROFILE OF HONEYS FROM STINGLESS BEES

Most of the physical-chemical profiles were performed with bees occurring in Brazil, and the most studied honey of native bees is that from bees of the genus *Melipona*, as can be seen in Table I. Analyses of honey from *Melipona scutellaris* harvested in different locations: Brejo Paraibano region, northeastern Brazil (Evangelista-Rodrigues et al. 2005), in Bahia (Souza et al. 2009b), Paraná (Nascimento et al. 2015), and in Santa Catarina (Biluca et al. 2016) showed that there is some variation in the content of hydroxymethylfurfural (HMF) between samples; this parameter indicates deterioration levels of honey. Taking as a reference the limits established in the standards applied to *A. mellifera* honey, the values obtained for the samples of *M. scutellaris* honeys would be in conformity for the free acidity index, diastase activity, HMF, sucrose, and ash content.

Regarding the level of free acidity of honey from the genus *Melipona*, the honey from *Melipona flavolineata*, harvested in Brazil, had the highest index, whose value was 143.67 meq/kg (Lemos et al. 2017), while *Melipona quadrifasciata anthidioides*, produced honey with an index of 17 meq/kg, also harvested in Brazil (Duarte et al. 2018). For the dehumidified honey of *Melipona quadrifasciata*, an index of 7.5 meq/kg was obtained (Carvalho et al. 2009). Of the total, 27% of the samples evaluated are above the required standard due to the fermentation that occurs naturally in honey from stingless bees, so this parameter is not recommended for use in assessing the quality of honey from stingless bees.

Melipona asilvai honey, although harvested in the Northeastern region of Brazil, has significant differences in HMF content, conductivity, and acidity (Souza et al. 2004; Souza et al. 2009a; Duarte et al. 2018). The exact period of the harvest and analysis of these samples must be considered in order to justify the differences.

Comparing the data in the literature (Table I) with the legislation for *A. mellifera*, it can be seen that the vast majority of the data obtained in the studies exceed the standard limits established for *Apis mellifera* honey, where the high moisture and acidity (Brazil 2000), humidity and free acidity are notable (Codex Alimentarius Commission 2001). Ash, sucrose, HMF contents, and diastase activity are less problematic, but eventually samples are outside the established limits. Therefore, the future regulations should review the standard limits to fit the majority of studied stingless bee honeys.

The laws of the state of São Paulo, Bahia, and Amazonas, which are aimed at regulating the quality of Meliponini honeys, represent a great advance in this issue in Brazil. There are still some gaps that need to be addressed, and probably changed in the future, but they are already allowing stingless bee keepers and honey industries to sell Meliponini honey in the official market. One of the parameters that should be revised is the free acidity levels. São Paulo and Bahia laws kept the same limit established for *Apis mellifera* honey (50 meq/kg) and Amazonas increased the tolerance to 80 meq/kg. This parameter is used to evaluate if the honey of apiculture industry has fermented. Because of natural fermentation that occurs in Meliponini honey (Menezes et al. 2013), it does not make much sense for stingless bee industry. About 30% of the samples are above the limit of 50 meq/kg and eventually higher than 80 meq/kg. For the content of reducing sugars, the

Table I. Physico-chemical analysis honey samples from native stingless bees

Species	Physico-chemical parameters												References
	Honey	Acidity (meq/kg)	Reducing sugars (g/100 g)	Diastatic activity (Gothe)	Conductivity (mS/cm)	HMF (mg/kg)	pH	Protein (%)	Sucrose (g/100 g)	Ash (g/100 g)	Moisture (g/100 g)	Country	
Cephalotrigona capixaba	*In natura*	34.33	75.21	0.18	0.73	35.4	3.04	-	0.36	0.19	32.1	Brazil	Nascimento et al. (2015)
Friesecomelitta doederleini	*In natura*	27.4	49.1	-	-	-	3.5	-	5.1	1.1	17.3	Brazil	Sousa et al. (2013)
F. doederleini	*In natura*	93.50	60.15	-	6.19	4.33	3.82			0.22	27.12	Brazil	Santisteban et al. (2019)
F. flavicornis	*In natura*	31.6	49.7	-			3.5	0.1	5.9	0.3	27.9	Brazil	Sousa et al. (2013)
F. varia	*In natura*	28.8	75	19.1	1.2	28	5.2	–	-	-	30	Brazil	Duarte et al. (2018)
	In natura	73	61	7.8	-	1.1		1.34	4.8	0.76	19.9	Venezuela	Vit et al. (1994)
Geniotrigona thoracica	*In natura*	-	-	-	-	-	3.36	0.96	-	-	28.17	Malaysia	Abu Bakar et al. (2017)
Heterotrigona itama	*In natura*	-	-	-	-	-	3.32	2.8	-	0.44	28.43	Malaysia	Abu Bakar et al. (2017)
Homotrigona fimbriata	*In natura*	528	22.4	-	2.6	46	3.3	-	-	1	41	Tailandia	Chuttong et al. (2016)
Hypotrigona sp.	*In natura*	35.57	60.49	-	0.3	-	3.75	5.74	1.83	-	17.5	Nigeria	Nweze et al. (2017)
Lepidotrigona doipaensis	*In natura*	197.5	27.8	1.7	1.19	2.3	3.5	-	-	0.66	32	Thailand	Chuttong et al. (2016)
L. flavibasis	*In natura*	168	29	3.1	1.3	8.5	3.7	-	-	0.51	28	Thailand	
L. terminata	*In natura*	194	13	0.29	0.78	-	3.5	-	-	0.24	30	Thailand	
Listotrigona fuerva	*In natura*	53	60	-	0.34	0.21	3.9	-	-	0.32	28	Thailand	
Melipona rufiventris mondory	*In natura*	38.2	65.6	<3	0.25	<L	4.21	-	<L	-	27.7	Brazil	Biluca et al. (2016)
M. asilvai	*In natura*	54.23	61.26	-	5.47	14.71	3.55	0.33	3.34	0.09	37.53	Brazil	Souza et al. (2009a)
	In natura	22	67	2	0.3	61	4.3		-	-	30	Brazil	Duarte et al. (2018)
	In natura	41.64	73.84	-	3.63	2.44	3.27	-	4.7	-	29.49	Brazil	Souza et al. (2004)
M. beecheii	*In natura*	41.52	–	1.3	0.58	9.23	3.2	2.71	-	0.46	28.62	Cuba	Alvarez-Suarez et al. (2018)
M. bicolor	*In natura*	91.62	60.14	<3	0.58	<L	3.77	-	<L	-	34.68	Brazil	Biluca et al. (2016)
	In natura	48.58	68.43	0.12	0.54	31.58	3.32	-	0.57	0.18	36.18	Brazil	Nascimento et al. (2015)
M. capixaba	*In natura*	79.28	-	-	-	-	3.62	-	-	-	30.51	Brazil	Lage et al. (2012)
M. compressipes	*In natura*	23.88	60.39	-	-	-	3.74	-	0.14	-	26.7	Brazil	de Almeida-Muradian and Matsuda (2007)
	In natura	48.4	75.7	1.1	-	1		0.49	1.6	0.3	23.4	Venezuela	Vit et al. (1994)
M. compressipes fasciculata	*In natura*	37.8	52.7	-	-	-	4.1	0.1	5.4	0.1	29.6	Brazil	Sousa et al. (2013)
M. crinita	*In natura*	-	-	-	-	-	-	-	-	-	28.8	Venezuela	Rodriguez-Malaver et al. (2009)
M. eburnea	*In natura*	-	-	-	-	-	-	-	-	-	23.8	Venezuela	Rodriguez-Malaver et al. (2009)
M. fasciculata	*In natura*	18.91	70.57	-	-	17.81	4.56	-	2.17	-	29.03	Brazil	Lemos et al. (2017)
	In natura	17.64	63.47	-	-	6.54	-	-	3.89	-	24.33	Brazil	Menezes et al. (2018)
	Pasteurized	16.08	63.83	-	-	9.46	-	-	1.59	-	23.68	Brazil	
M. favosa	*In natura*	62.9	72.1	0.9	-	1.2	-	0.41	1.5	0.29	25.5	Venezuela	Vit et al. (1994)

Table 1 (continued)

Species	Physico-chemical parameters											References
	Honey	Acidity (meq/kg)	Reducing sugars (g/100 g)	Diastatic activity (Gothe)	Conductivity (mS/cm)	HMF (mg/kg)	pH	Protein (%)	Sucrose (g/100 g)	Ash (g/100 g)	Moisture (g/100 g)	Country	
	In natura	36.8	70.3	2.86	2.06	17.1	-	0.71	2	0.15	24.2	Venezuela	Vit et al. (1998)
M. flavolineata	*In natura*	143.67	59.31	-	-	34.62	3.41	-	5.52	-	35.11	Brazil	Lemos et al. (2017)
	In natura	38.85	63.09	-	-	3.59		-	2.12	-	28.53	Brazil	Menezes et al. (2018)
	Pasteurized	32.46	62.70	-	-	43.10		-	1.62	-	27.40	Brazil	
M. grandis	*In natura*	-	-	-	-	-	-	-	-	-	27.5	Venezuela	Rodriguez-Malaver et al. (2009)
M. ilota	*In natura*	-	-	-	-	-	-		-	-	28	Venezuela	Rodriguez-Malaver et al. (2009)
M. lateralis kangarumensis	*In natura*	40.7	64.8	2.76	1.65	3.9		0.23	1.1	0.11	28.8	Venezuela	Vit et al. (1998)
M. mandacaia	*In natura*	43.48	74.82	-	3.52	5.79	-	-	2.91	-	28.78	Brazil	Alves et al. (2005)
	In natura	37.7	75.5	-	2.84	30.85	3.71	0.17	2.85	0.09	31.4	Brazil	Souza et al. (2009a)
M. marginata	*In natura*	22.55	67.39	0.19	0.62	48.09	2.93	-	0.85	0.14	32.44	Brazil	Nascimento et al. (2015)
	In natura	79.82	63.5	< 3	0.44	<L	3.67	-	<L	-	32.65	Brazil	Biluca et al. (2016)
M. mondury	*In natura*	61.51	-	-	-	-	4.19	-	-	-	-	Brazil	Lage et al. (2012)
	In natura	37.89	67.77	0.2	0.51	51.38	3.5	-	0.85	0.25	29.97	Brazil	Nascimento et al. (2015)
	In natura	61.1	67.45	< 3	0.69	<L	5.18	-	<L	-	29.75	Brazil	Biluca et al. (2016)
M. paraensis	*In natura*	30.4	60.8	2.9	1.37	3.4		0.14	1.2	0.14	26.4	Venezuela	Vit et al. (1998)
M. q. anthidioides	*In natura*	17	75	3	0.5	33	4.2	-	-	-	31	Brazil	Duarte et al. (2018)
M. quadrifasciata	*In natura*	33.5	52.8	-	-	-	3.8	0.2	6.6	0.58	28.1	Brazil	Sousa et al. (2013)
	In natura	42.52	61.77	11.25	0.33	<L	3.71	-	<L	-	32.47	Brazil	Biluca et al. (2016)
	In natura	35	71.63	0.13	0.58	42.63	3.18	-	0.85	0.16	36.89	Brazil	Nascimento et al. (2015)
	Dehumidified	7.5	72.99	1.34	5.97	3.82	6.04	-	2.52	0.41	16.9	Brazil	Carvalho et al. (2009)
	In natura	6.27	60.42	1.40	4.46	1.27	6.64	-	1.28	0.39	25.20	Brazil	
	Dehumidified	28.5	74.63	1.65	2.17	4.39	3.46	-	2.96	0.12	16.90	Brazil	
	In natura	28.0	60.06	2.14	2.26	1.45	3.74	-	1.32	115	> 30.0	Brazil	
M. quadrifasciata anthidioides	*In natura*	40.8	68.29	-	5.49	16.04	3.99	0.29	3.09	0.1	32.09	Brazil	Souza et al. (2009a)
M. quinquefasciata	*In natura*	36.9	64	-	-	-	3.5	0.3	5.8	0.1	28.8	Brazil	Sousa et al. (2013)
M. rufiventris	*In natura*	42	-	-	-	-	4.24	-	-	-	-	Brazil	Lage et al. 2012
M. scutellaris	*In natura*	28.33	-	-	-	18.92	4.66	-	-	0.17	25.26	Brazil	Evangelista-Rodrigues et al. 2005
	In natura	27.25	66.41	0.11	0.54	40.86	3.48	-	0.7	0.16	33.98	Brazil	Nascimento et al. (2015)
	In natura	28.7	62.7	< 3	0.15	<L	4.52	-	<L	-	23.4	Brazil	Biluca et al. (2016)
	In natura	37	59	2	0.7	21	4.2	-	-	-	30	Brazil	Duarte et al. (2018)
	Dehumidified	53.5	67.57	1.73	2.9	6.65	3.71	-	1.51	0.18	16	Brazil	Carvalho et al. (2009)
	In natura	55.0	53.91	2.16	2.72	2.21	3.53	-	1.11	0.18	> 30.0	Brazil	
	Dehumidified	28.5	70.92	2.18	2.72	3.02	3.67	-	4.35	0.17	16.5	Brazil	

Table I (continued)

Species	Physico-chemical parameters												References
	Honey	Acidity (meq/kg)	Reducing sugars (g/100 g)	Diastatic activity (Gothe)	Conductivity (mS/cm)	HMF (mg/kg)	pH	Protein (%)	Sucrose (g/100 g)	Ash (g/100 g)	Moisture (g/100 g)	Country	
	In natura	27.75	56.98	3.01	2.64	1.33	3.57	-	3.17	0.18	26.0	Brazil	
	In natura	86.2	49.8	-	-	-	4.1	1.8	5.3	0.1	35.4	Brazil	Sousa et al. (2013)
	In natura	19.87	70.71	-	5.7	1.99	4.43	0.25	1.81	0.19	29.13	Brazil	Souza et al. (2009a)
M. seminigra	*In natura*	26.54	61.49	-	-	-	3.78	-	0.18	-	30.4	Brazil	de Almeida-Muradian and Matsuda (2007)
	In natura	30.44	69.12	0.2	0.55	29.5	3.72	-	1.61	0.22	27.85	Brazil	Nascimento et al. (2015)
Melipona sp.	*In natura*	35.7	49.4	15.63	3.92	8.6	3.6	0.23	3.8	0.38	38.7	Brazil	do Vale et al. (2018)
Melipona sp.	*In natura*	12.59	75.64	-	0.24	5.5	4.21	5.5	5.06	-	13.86	Nigeria	Nweze et al. (2017)
M. subnitida	*In natura*	32.49	-	42.87	1.03	7.56	-	0.28	4.86	0.02	24.8	Brazil	Almeida-Muradian et al. (2013)
	In natura	22	75	3	0.6	51	4.6	-	-	-	27	Brazil	Duarte et al. (2018)
	In natura	38.1	52.6	-	-	-	4.4	0.9	3.7	0.2	31.1	Brazil	Sousa et al. (2013)
	Heated	39.1	80.94	-	-	56.53	3.8	-	-	-	25.17	Brazil	Freitas et al. (2010)
M. triplasis	*In natura*	24.24	76.66	1	-	1.3		-	1.48	0.12	25.7	Venezuela	Vit et al. (1994)
	In natura	24.2	73.7	1	-	1.3		0.48	1.5	0.12	25.7	Venezuela	Vit et al. (1998)
Nannotrigona testaceicornis	*In natura*	64.5	42.2	-	-	-	5	1.7	10.2	0.6	34.7	Brazil	Sousa et al. (2013)
Plebeia sp.	*In natura*	125	72	10	1.2	72	4.1	-	-	-	35	Brazil	Duarte et al. (2018)
Scaptotrigona bicunctata	*In natura*	48.95	62.95	4.34	0.63	<L	4.48	-	<L	-	23.95	Brazil	Biluca et al. (2016)
S. mexicana	*In natura*	68.3	56.48	-	0.4	16.4	3.82	-	-	0.45	20.61	Mexico	Jimenez et al. (2016)
Scaptotrigona sp.	*In natura*	35.6	46.3	-	-		5.1	0.4	1.5	0.3	28.3	Brazil	Sousa et al. (2013)
Scaptotrigona sp.	*In natura*	60.98	62.34	-	-	24.71	3.89	-	4.83	-	30.22	Brazil	Lemos et al. (2017)
S. xanthotricha	*In natura*	28.78	66.32	0.62	0.62	58.27	3.58	-	1.22	0.21	29.84	Brazil	Nascimento et al. (2015)
S. hipnorata	*In natura*	38.57	60.01			4.85	3.97		4.65	0.36	19.07	Brazil	Oliveira e Santos (2011)
Tetragona carbonária	*In natura*	128.9	42	0.4	1.64	1.2	4	2.02	1.8	-	16.4	Australia	Persano Oddo et al. (2008)
Tetragona clavipes	*In natura*	91.2	48.6	19.1	1.01	<L	4.28	-	<L	-	25.2	Brazil	Biluca et al. (2016)
Tetragonilla collina	*In natura*	25	52	0.34	0.43	5.9	3.7	-	-	0.24	28	Thailand	Chuttong et al. (2016)
Tetragonisca angustula	*In natura*	45.23	55.46	32.28	1.34	9.39	4.1	0.37	0.95	0.39	24.37	Brazil	Anacleto et al. (2009)
	In natura	27	66.65	22.43	0.72	27.99	4.08	-	0.82	0.33	25.99	Brazil	Nascimento et al. (2015)
	In natura	41.15	63.75	49.6	0.95	<L	4.77	-	<L	-	23.75	Brazil	Biluca et al. (2016)
	In natura	48.3	65.9	23	7.32	9.8		1.42	2.1	0.38	23.2	Venezuela	Vit et al. (1998)
	In natura	79	43	-	-	-	3.72	-	-	-	24	Argentina	Pucciarelli et al. (2014)
	In natura	39.2	53.6	16.7	0.66	1.3	4.2	-	4.2	0.21	24.3	Colombia	Fuenmayor et al. (2012)
	Dehumidified	68.25	76.92	4.05	-	53.89	3.83	-	3.85	-	17.5	Brazil	Alves et al. (2012)
Tetragonisca clavipes	*In natura*	59	72	9	1.4	18	5.6	-	-	-	19	Brazil	Duarte et al. (2018)
Tetragonula fuscobalteata	*In natura*	96.5	32.7	4.7	1.35	22	3.6	-	-	0.67	26	Thailand	Chuttong et al. (2016)

Table I (continued)

Species	Physico-chemical parameters												References
	Honey	Acidity (meq/kg)	Reducing sugars (g/100 g)	Diastatic activity (Gothe)	Conductivity (mS/cm)	HMF (mg/kg)	pH	Protein (%)	Sucrose (g/100 g)	Ash (g/100 g)	Moisture (g/100 g)	Country	
Tetragonula laevipes	*In natura*	81.37	47.87		0.62	1.07	3.62	-	-	0.26	26.98	Thailand	Suntiparapop et al. (2015)
Tetragonula laevipes-pagdeni complex	*In natura*	76	-	0.63	0.59	5.4	3.6	-	0.03	0.22	28	Thailand	Chuttong et al. (2016)
Tetragonula testaceitarsis	*In natura*	70.5	41	0.22	0.59	2.4	3.2	-	-	0.21	30.5	Thailand	
Tetrigona apicalis	*In natura*	495	12.65	4.9	2.6	0.26	3.4	-	-	1.4	42	Thailand	
Tetrigena melanoleuca	*In natura*	592	7.45	0.15	2.8	28	3.6	-	-	3.1	43	Thailand	
Trigona fuscipennis	*In natura*	46.7	56.6	< 3	0.31	<L	3.44	-	<L	-	34.4	Brazil	Biluca et al. (2016)
Trigona sp.	*In natura*	78.14	29.34	16.67	0.57	3.18	3.35	-	-	0.2	13.26	Thailand	Issaro et al. (2013)
Trigonadaeviceps Smith	*In natura*	50.83	27.37	13.64	0.57	3.32	3.44	-	-	0.14	15.73	Thailand	
Trigonapagdenis Schwarz	*In natura*	20	41.64	11.11	0.45	3.97	4.01	-	-	0.22	14.66	Thailand	
Amazonas State Regulations	Dehumidified	80 max	50 min	3 max	-	40 max	-	-	6 max	0.6 max	22 max	Brazil	Brazil (2016)
	Chilled	80 max	50 min	3 max	-	40 max	-	-	6 max	0.6 max	23–35 max	Brazil	
	In Natura	80 max	50 min	3 max	-	40 max	-	-	6 max	0.6 max	23–35 max	Brazil	
São Paulo State Regulations	Dehumidified	50 max	60 min	-	-	20 max	2.9–4.5	-	6 max	0.6 max	20 max	Brazil	Brazil (2017)
	Pasteurized	50 max	60 min	-	-	20 max	2.9–4.5	-	6 max	0.6 max	40 max	Brazil	
	Matured	50 max	60 min	-	-	20 max	2.9–4.5	-	6 max	0.6 max	40 max	Brazil	
	In Natura	50 max	60 min	-	-	20 max	2.9–4.5	-	6 max	0.6 max	40 max	Brazil	
Bahia State Regulations	Chilled	50 max	60 min	3 max	-	10 max	-	-	6 max	0.6 max	20–30 max	Brazil	Brazil (2014)
	Dehumidified	50 max	60 min	3 max	-	10 max	-	-	6 max	0.6 max	19 max	Brazil	
Legislation IN n° 11	-	50 max	65 min	8 min	-	60 max	-	-	6 max	1.2 max	20 max	Brazil	Brazil (2000)
International	-	50 max	60 min	-	-	60 max	-	-	5 max	0.6 max	20 max	-	Codex Alimentarius Commission (2001)

<*L* limit of quantification

value determined by these regulations is less than 50 g/100 g. For the moisture level, it can be observed in the regulations that the levels required for fresh and chilled honey are allowed to reach a maximum of 35 g/100 g, while the proposals allow for up to 40 g/100 g. These differences may be associated with the floral origin of the honeys.

When comparing the physico-chemical parameters determined with European (Codex Alimentarius Commission 2001) and Brazilian legislation (Brazil 2000), the acidity index, reducing sugars, diastase activity, HMF, and humidity are the ones that present quite different values from those established. Of the 106 studies listed in Table I, 29 had acidity levels above the reference value, 31 contained levels lower than those established for reducing sugars, over 33 had diastase activity below what is permitted by legislation, 12 had HMF levels higher than established levels, and 82 humidity levels were above those stipulated. For sucrose and ash contents, in the vast majority of studies, the values are in accordance with legal limits. When the comparison takes place with what the Brazilian states of Amazonas, São Paulo, and Bahia define as standard, we can observe that this quantity is smaller, only 5 samples of honey provided values above those stipulates for acidity levels, for reducing sugars, 15 of them did not meet the regulations, for diastase activity, even though the proposals establish a maximum of 3 on the Gothe scale, 27 presented higher values. For the HMF content, 10 provided values above the regulations and for humidity, only 2 honey samples were not in compliance. Analyzing the data, it can be noted that what makes the honey of native bees and *Apis mellifera* considerably different are not only the high levels of acidity and humidity but also other factors as well. For example, the content of reducing sugars and diastase activity shows significant discrepancies from those stipulated for non-stingless bee honey.

Almeida-Muradian et al. (2013) studied samples of honeys from *Apis mellifera* and *Melipona subnitida* and found that the honey of *A. mellifera* showed values within the established limits, while that of the stingless bee presented values for diastase activity 5 times greater than the minimum stipulated, and the honey moisture was also slightly above the norm (Table I). The results of the palynological analysis showed that even though they were subjected to the same flora, bees of different species access different plant sources.

Table I also includes data from different methods used in the treatment of honey. The expressive moisture content creates product instability over time, as it is very susceptible to fermentation. To overcome the problems arising from this, good harvesting practices are necessary, and these should aim at reducing contamination by microorganisms. Once harvested, some processing methods can be applied to assist in the conservation of this product. These are refrigeration, dehumidification, pasteurization, and maturation (Venturieri et al. 2007; Contrera et al. 2011).

Freitas et al. (2010) used heating in order to evaluate the physico-chemical parameters of the honey from *Melipona subnitida* in its natural form. After heating in an oven at 70 °C for different periods, the results indicated that the heat treatment decreased the acidity and humidity; however, the content of HMF and reducing sugars increased significantly. When compared with regulation proposals, with the exception of the HMF content, the other parameters were in accordance with the established levels.

Alves et al. (2012) evaluated the physico-chemical and sensory stability of the dehumidified honey of *Tetragonisca angustula*. The results showed good physico-chemical stability for the parameters of humidity, reducing sugars, apparent sucrose, pH, acidity, and HMF during a storage period of 180 days. However, only the pH and humidity corresponded to the values established in the regulation proposal by Camargo et al. (2017) for dehumidified honey. In comparison with the regulations of the Amazonas state, the parameters for acidity, reducing sugars, and humidity are in accordance.

Menezes et al. (2018) adopted pasteurization as a measure to minimize the proliferation of microorganisms in the honey from *M. fasciculata* and *M. flavolineata*. The process significantly influenced the moisture, pH, apparent sucrose, and HMF of the honeys, but did not influence acidity, ash, and reducing sugars.

The results show that when the moisture content of honey from stingless bees is adjusted, the

other parameters are altered. Therefore, there is no treatment that meets the peculiarities of the honey produced by these bees nor does it make any treatment universally suitable for quality parameters. Thus, proposed regulations already admit specific values for the parameters in the different forms of processing. Carvalho et al. (2009) studied Melipona honeys which had been harvested in different places and subjected to the dehumidification process. The honey produced by *M. quadrifasciata*, which was harvested on the island of Itapara and compared *in natura* with dehumidified form, showed an increase in the acidity index, reducing sugars, HMF, and sucrose, and a decrease in the diastase activity values, pH, and, consequently, humidity. These changes are compatible with what is expected for this treatment. This profile was also observed in the samples from Costa do Sauipe, Bahia State, Brazil. The honeys showed relevant differences for some parameters, such as the total acidity index which was lower for honey harvested on the Island of Itapara. For this honey, the pH is lower than for the honey harvested on the Costa do Sauipe. In relation to the honey from *M. scutellaris*, honey harvested in Tucano showed alterations in the content of sucrose and HMF, and showed an increase after the treatment, whereas the one harvested in Serrinha presented an increase in the levels of sucrose, HMF, and reducing sugars. The values for most of the evaluated parameters are in accordance with those established in the proposals and in the regulations of the state of Amazonas.

The studies also gather a significant amount of data on the honeys of species of the genera *Scaptotrigona* and *Tetragonisca*, and for the later of these two genus, seven of the eight studies were carried out with *T. angustula* species.

4. METABOLITES AND BIOLOGICAL ACTIVITY OF HONEYS FROM STINGLESS BEES

The literature has little data regarding the chemical composition of honey from stingless bees, and studies tend to focus on the quantification of phenolics compounds and flavonoids. These determinations are supported by positive correlations between the presence of these compounds with antioxidant activity (Table II).

The phenolic content was the most commonly determined parameter in the honeys studied so far (Table II). The phenolic content of *M. subnitida* was 0.6 mg AGEq 100 g^{-1} in honey from the state of Amazonas (Brazil) and 854.62 mg AGEq 100 g^{-1} in honey from the state of Sergipe (Brazil). Other studies with other species have also revealed different phenolic contents for *M. fasciculata* and *M. flavolineata* (Oliveira et al. 2012), as well as for *M. s. merrillae* (Silva et al. 2013). These differences in content for the same species indicate that it will be difficult to create an adequate parameter for quality, since the flora is very diverse. Perhaps the way forward is to create a geographic seal after monitoring the parameters at different times of the year and for several years.

Different methods were used to determine the antioxidant capacity of stingless bee honeys, with DPPH and ABTS free radical scavenging being the most commonly used. When the data in Table II for the activity using these two methods is analyzed, it appears that the values vary widely.

Ávila et al. (2019) evaluated the antioxidant action of meliponine honeys using three different methods and the ORAC method the values were more expressive.

The study by Duarte et al. (2018) with 31 samples of meliponine honey from the same meliponary, in the state of Alagoas, Brazil, describes the differences in the content of phenolic compounds and flavonoids, which suggests preferences for different types of nectar.

For the honey of *M. s. merrillae* harvested in different locations in the state of Amazonas, differences in phenolic content were observed. Samples obtained in Pauíni and Maués showed the highest levels: 64.0 ± 0.03 and 66.0 ± 0.05 mgAGEq 100 g^{-1}, respectively. However, these samples were expected to have better antioxidant activity, but compared with those with lower concentrations, there was no significant difference (Silva et al. 2013). The authors also carried out the characterization of this stingless bee honey and detected by means of high-performance liquid chromatography (HPLC) the presence of 14 phenolic compounds in the ethyl acetate fraction. The presence of some of the

Table II. Phenolic and flavonoid content and antioxidant activity in honeys from stingless bees

Species	Phenolics (mgAGE GAEq 100 g^{-1})	Flavonoids (mgQEq 100 g^{-1})	Antioxidant activity	
			DPPH	ABTS
Frieseomelitta doederleini	1.19 ± 0.06	0.16 ± 0.02	13.38 mg mL^{-1}	4.94 mg mL^{-1}
F. varia	89.2	29.2	-	-
Hypotrigona sp.	52.74 ± 3.60	4.14 ± 10.65	-	-
Melipona bicolor	22.04–70.8	n.d.	9.71–33.49 (μmolTE/kg)	1.61–23.73 (μmolTE/kg)
M. eburnea	n.d.	8.9 ± 0.7	-	206.0 ± 9.9 μmoles TE/100 g
M. flavolineata	26.39	n.d.	48.92 mg 100 g	-
	236.71	n.d.	6.85 mg 100 g	-
	56.78	n.d.	32.03 mg 100 g	-
M. fasciculata	25.53	n.d.	54.43 mg 100 g	-
	88.81	n.d.	15.58 mg 100 g	-
	59.78	n.d.	31.04 mg 100 g	-
M. grandis	n.d.	3.1 ± 1.3	-	107.0 ± 17.3 μmoles TE/100 g
M. marginata	25.4–41.4		12.44–18.23 (μmolTE/kg)	7.34–13.67 (μmolTE/kg)
M. s. merrillae	26.5 ± 0.05	n.d.	-	0.3 ± 0.01 μg mL^{-1}
	17.0 ± 0.02	n.d.	-	0.3 ± 0.02 μg mL^{-1}
	64.0 ± 0.03	n.d.	-	0.2 ± 0.03 μg mL^{-1}
	34.0 ± 0.01	n.d.	-	0.2 ± 0.01 μg mL^{-1}
	43.0 ± 0.02	n.d.	-	0.2 ± 0.02 μg mL^{-1}
	36.0 ± 0.01	n.d.	-	0.2 ± 0.03 μg mL^{-1}
	66.0 ± 0.05	n.d.	-	0.2 ± 0.01 μg mL^{-1}
M. anthidioides	78 ± 48	45 ± 37	-	-
M. asilvai	32 ± 9	8 ± 2	-	-
	82.91 ± 1	79.73 ± 1.6	41.33 ± 0.9 mg/mL	-
M. beecheii	94.39 ± 14.55	4.19 ± 0.37	42.23 ± 1.66 μg mL^{-1}	-
M. compressipes	30.71 ± 2.01	44.63 ± 2.3	37.79 ± 1.2 mg/mL	-
M. crinita	n.d.	7.3 ± 0.6	-	237.4 ± 13.1 μmoles TE/100 g
M. illota	n.d.	2.6 ± 0.1	-	93.8 ± 10.1 μmoles TE/100 g
M. mandacaia	61.72 ± 1.1	45.42 ± 2	28.1 ± 0.6 mg/mL	-
M. q. anthidioides	161.8 ± 3.4	43.09 ± 2	40.03 ± 0.4 mg/mL	-
M. q. quadrifasciata	82.19 ± 1.2	75.45 ± 2.71	25.39 ± 0.5 mg/mL	-
M. quadrifasciata	31.5–58.5	n.d.	18.12–26.95 (μmolTE/kg)	2.63–31.32 (μmolTE/kg)
M. scutellaris	62 ± 15	29 ± 14		-
M. scutellaris	192.01 ± 2.8	30.24 ± 2	51.44 ± 0.7 mg/mL	-

Table II (continued)

Melipona sp.	37.20 ± 14.18	8.64 ± 4.69	-		-
M. subnitida	38 ± 11	11 ± 2	-		-
M. subnitida	0.6	n.d.	5.9 μg mL^{-1}		-
M. subnitida	854.62 ± 3.8	279.73 ± 4.6	37.69 ± 1 mg/mL		-
Nannotrigona melanocera	n.d.	31.0 ± 1.2	-		569.6 ± 7.3 μmoles TE/100 g
Partamona epiphytophila	n.d.	5.9 ± 0.3	-		115.7 ± 3.5 μmoles TE/100 g
Plebeia sp.	104 ± 20	35 ± 13	-		
Ptilotrigona lurida	n.d.	23.4 ± 1.1	-		205.7 ± 11.3 μmoles TE/100 g
Scaptotrigona bipuncata	27.7–66.1	n.d.	14.61–39.10 (μmolTE/kg)		11.35–34.73 (μmolTE/kg)
S. mexicana	25.85–40.1	n.d.	15.65–19.04%		
S. polystica	n.d.	17.6 ± 0.7	-		330.2 ± 14.8 μmoles TE/100 g
Scaura latitarsis	n.d.	17.7 ± 0.9	-		255.8 ± 5.0 μmoles TE/100 g
Tetragona clavipes	136 ± 32	55 ± 20	-		
Tetragonisca angustula	n.d.	18.8 ± 0.7	-		327.7 ± 2.9 μmoles TE/100 g
Trigona sp.	22.81 ± 7.9	9.79 ± 10.1	-		

Species	Antioxidant activity			Country	References
	FRAP	β- Carotene/ linoleic acid	ORAC		
Frieseomelitta doederleini	-	-	-	Brazil	Santisteban et al. (2019)
F. varia	-	-	-	Brazil	Duarte et al. (2018)
Hypotrigona sp.	666.88 ± 1.73 μmol Fe(II)/100 g	-	-	Nigeria	Nweze et al. (2017)
Melipona bicolor	-	-	48.05–79.11 (μmolTE/kg)	Brazil	Ávila et al. (2019)
M. eburnea	-	-	-	Peru	Rodríguez-Malaver et al. (2009)
M. flavolineata	-	-	-	Brazil	Oliveira et al. (2012)
	-	-	-	Brazil	
	-	-	-	Brazil	
M. fasciculata	-	-	-	Brazil	
	-	-	-	Brazil	
	-	-	-	Brazil	
M. grandis	-	-	-	Peru	Rodríguez-Malaver et al. (2009)
M. marginata	-	-	47.49–82.87 (μmolTE/kg)	Brazil	Ávila et al. (2019)

Table II (continued)

M. s. merrillae	-	-	-	Brazil	Silva et al. (2013)
	-	-	-	Brazil	
	-	-	-	Brazil	
	-	-	-	Brazil	
	-	-	-	Brazil	
	-	-	-	Brazil	
	-	-	-	Brazil	
M. anthidioides	-	-	-	Brazil	Duarte et al. (2018)
M. asilvai	-	-	-	Brazil	
	-	-	-	Brazil	Oliveira et al. (2017)
M. beecheii	38.54 ± 11.37 μmol Fe(II)/100 g	-	-	Cuba	Alvarez-Suarez et al. (2018)
M. compressipes	-	-	-	Brazil	Oliveira et al. (2017)
M. crinita	-	-	-	Peru	Rodríguez-Malaver et al. (2009)
M. illota	-	-	-	Peru	Rodríguez-Malaver et al. (2009)
M. mandacaia	-	-	-	Brazil	Oliveira et al. (2017)
M. q. anthidioides	-	-	-	Brazil	
M. q. quadrifasciata	-	-	-	Brazil	
M. quadrifasciata	-	-	49.29–94.35 (μmolTE/kg)	Brazil	Ávila et al. (2019)
M. scutellaris	-	-	-	Brazil	Duarte et al. (2018)
M. scutellaris	-	-	-	Brazil	Oliveira et al. (2017)
Melipona sp.	426.93 ± 11.55 μmol Fe(II)/100 g	-	-	Nigeria	Nweze et al. (2017)
M. subnitida	-	-	-	Brazil	Duarte et al. (2018)
M. subnitida	-	-	-	Brazil	Bastos et al. (2009)
M. subnitida	-	-	-	Brazil	Oliveira et al. (2017)
Nannotrigona melanocera	-	-	-	Peru	Rodríguez-Malaver et al. (2009)
Partamona epiphytophila	-	-	-	Peru	
Plebeia sp.	-	-	-	Brazil	Duarte et al. (2018)
Ptilotrigona lurida	-	-	-	Peru	Rodríguez-Malaver et al. (2009)
Scaptotrigona bipuncata	-	-	35.49–85.48 (μmolTE/kg)	Brazil	Ávila et al. (2019)
S. mexicana	50.42–61.10 μmolTE/100 g	40.45–70.45%	-	Mexico	Jimenez et al. (2016)
S. polystica	-	-	-	Peru	Rodríguez-Malaver et al. (2009)

Table II (continued)

Scaura latitarsis	-	-	-	Peru		
Tetragona clavipes	-	-	-	Brazil	Duarte et al. (2018)	
Tetragonisca angustula	-	-	-	Peru	Rodríguez-Malaver et al. (2009)	
Trigona sp.	-	-	-	Malaysian	Ranneh et al. (2018)	

GAE gallic acid equivalent, *QE* quercetin equivalents, *TE* trolox equivalents, *ORAC* oxygen radical absorbance capacity, n.d: not determined

[a] DPPH capacity of scavenge the 2,2-diphenyl-1-picryhydrazyl free radical

[b] ABTS 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical activity

[c] FRAP reducing power of iron(III)/ferricyanide complex

constituents coincided with the honeys from the same floral source. The study reported the presence of the flavonoid taxifoline in honey from stingless bees and the presence of catechol in Brazilian honeys for the first time. In addition, some of the samples showed effective potential in inhibiting microbial growth.

Oliveira et al. (2012) carried out studies on honeys from the state of Pará (Brazil) and found differences in the phenolic content and antioxidant activity using DPPH on samples from different locations for the species *M. flavolineata* and *M. fasciculata*. These authors used high-performance liquid chromatography for identification by comparing with standards of compounds present in stingless bee honeys, and found differences in the composition with major constituents of quercetin and gallic acid.

Biluca et al. (2016) determined the content of phenolic compounds in samples of honeys from ten species of stingless bees; in different periods of the year, the variation in the contents of these compounds was justified by the difference in botanical origin; however, the quantification values are shown in graphs, the minimum values being 10.3 mg of gallic acid $100g^{-1}$ and maximum 98 mg of gallic acid $100g^{-1}$ of honey, found for the bees *Melipona quadifasciata* and *Tetragonisca angustula*, respectively. Biluca et al. (2017) identified and quantified the presence of mandelic acid, caffeic acid, chlorogenic acid, rosmarinic acid, aromadendrene, isoquercitrin, eriodictyol, vanillin, umbelliferone, syringaldehyde, synap aldehyde, and carnosol in native bee honeys and significant correlation of compounds with antioxidant activity expressed by these honeys. Alvarez-Suarez et al. (2018) identified 19 compounds in *M. beecheii* honey using HPLC-DAD-ESI MS/MS, among those identified were C-pentosyl-C-hexosyl-apigenin, coumaric acid, isorhamnetin, kaempferol, luteolin, apigenin, quercetin, ferulic acid, and dihydrocaffeic acid.

The aroma of honey, although it seems characteristic, is influenced by the great variety of volatile compounds from floral origin. In addition, several other factors can contribute to the "flavor" of honey, such as the bee's own physiology, as well as procedures after harvest in relation to the heating, storage, and other factors (Campos et al.

2000). Costa et al. (2018) analyzed honey from *Melipona subnitida* and *M. scutellaris* using extraction via HS-SPME and gas chromatography coupled with mass spectrometry and detected a total of 114 volatile compounds, of which the highest contents were terpenes, followed by esters, norisoprenoids, benzene derivatives, furans, ketones, hydrocarbons, alcohols, aldehydes, acids, in addition to a sulfur compound. Although the samples come from different plant origins, the presence of certain compounds in all honeys was noted, and others were detected in the samples of only one of the studied species. Compounds belonging to these classes have also been found in honey from *Apis mellifera* (Alissandrakis et al. 2007a, b; Alissandrakis et al. 2009; Anastasaki et al. 2009; Ceballos et al. 2010; Jerković et al. 2010a, b; Alissandrakis et al. 2011; Jerković et al. 2011a, b).

Silva et al. (2017) studied the composition of volatiles obtained by static headspace gas chromatography of eight species of bees native to the state of Paraná (Brazil) and identified 44 compounds, including derivatives of linalool, hotrienol, and esters, and attributed the composition to the geographical origin of the samples.

In addition to the anti-toxicity activity, other biological properties have also been investigated, due to the therapeutic use of honey produced by bees of the genus *Apis* and by stingless bees (Amin et al. 2018). The antimicrobial activity is the category that presents the most data, the honey of *Tetragonisca angustula* was the most commonly studied. Miorin et al. (2003) performed a microbial sensitivity test against *Staphylococcus aureus* and obtained a minimum inhibitory concentration that ranged from 142.87 to 214, 33 mg mL^{-1}, demonstrating an action lower than that of *Apis mellifera*, also evaluated in the study. Demera and Angert (2004) used agar diffusion and found that honey significantly inhibited the tested yeasts *Saccharomyces cerevisiae* (ATCC 287) and *Candida albicans* (ATCC 90028). Sgariglia et al. (2010) found similar results with growth inhibition of *Escherichia coli* (IEV301), *Pseudomonas aeruginosa* (IEV 305), *Staphylococcus aureus* (IEV7), *Staphylococcus aureus* (IEV 20), and *Enterococcus faecali* (IEV 208). Mercês et al. (2013) evaluated the antimicrobial action of honey from *T. angustula*, both by the agar diffusion method and by broth macrodilution, and only obtain activity against *S. aureus* and *E. coli* with a minimum inhibitory concentration equal to 28.2 mg mL^{-1} and 132 mg mL^{-1}, respectively.

For anti-tumor activity, the results indicate that honeys have significant action with different mechanisms on tumor cell lines (Vit et al. 2013). Kustiawan et al. (2014) evaluated extracts of honey from different species of native bees and all of them presented cytotoxicity on hepatoblastoma cells. Ahmad et al. (2019) induced apoptosis in malignant glioma cells for cytotoxic analysis of *Heterotrigone itama* honey. The results demonstrated cytotoxicity at certain periods and dosages, since honey induced nuclear shrinkage, chromatin condensation, and nucleus fragmentation. In addition to the cytotoxic action of honey, the investigation of its potential as a chemopreventive agent was carried out by Yazan et al. (2016), whose results showed that honey from *Trigona* sp. significantly reduced the total number of aberrant crypt foci, aberrant crypts, and multiplicity of colorectal crypts.

Regarding the anti-inflammatory effects, the studies by Borsato et al. (2014) and Ruiz-Ruiz et al. (2017) demonstrated different therapeutic effects that honey can have for this action. Borsato et al. (2014) evaluated the potential of *Melipona marginata* honey in reducing ear inflammation in test subjects and observed that the topical application of honey extract (1.0 mg/ear) was able to reduce ear edema. This extract decreased myeloperoxidase activity, which suggests a lower leukocyte infiltration and was confirmed by histological analysis. In addition, it also provided a reduction in the production of reactive oxygen species. Ruiz-Ruiz et al. (2017) carried out an in vitro determination using the evaluation of protein denaturation and observed that the flavonoid fraction of the methanol extract showed itself to be potent in inhibiting the denaturation of albumin and in membrane stabilization.

Ilechie et al. (2012) used different concentrations of fresh honey from *Meliponula* ssp. to treat bacterial conjunctivitis caused by *Staphylococcus aureus* or *Pseudomonas aeruginosa* induced in vivo in Hartley guinea pigs and found that the effect of honey was comparable with that of gentamicin, a standard antibiotic. In view of the

results, the authors suggest the use of honey as an alternative treatment for infections. Similar results were found by Kwapong et al. (2013) with *Meliponula bucandei* honey, which showed antimicrobial activity in vitro against bacteria isolated from eye infections (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*). The inhibitory effect of honey in reducing inflammation and infection was superior to the commonly used ophthalmic antibiotics.

Kwakman et al. (2010) suggest that the bioactive properties of honey are attributed to specific factors, such as the synergistic action of sugar and hydrogen peroxide for wound healing. In studies carried out with *Apis mellifera* honey, the samples that suffered a decrease in the accumulated H_2O_2 levels had a marked reduction in the antibacterial action against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* resistant strains. According to the authors, this indicates that H_2O_2 is important for the bactericidal activity of honey, but additional factors must also be present. For Yaghoobi et al. (2013), honey induces leukocytes to release cytokines, which initiate tissue repair cascades, in addition to activating the immune response to infection.

An outstanding finding was done recently by Fletcher et al. (2020) about the sugar composition of stingless bee honey. They found a high concentration of trehalulose, between 13 to 44 g per 100 g, in honey from five different stingless bee species across Neotropical and Indo-Australian regions. Trehalulose is a specific kind of disaccharide, considered to be beneficial for human health because of its acariogenic and low glycemic index properties. Besides, it is 70% as sweet as sucrose and not readily crystallized, therefore has commercial application in food industry (Fletcher et al. 2020).

5. FUTURE PERSPECTIVES

Significant differences are found between the stingless bee honey and *Apis mellifera* honey, as well as between the different species of stingless bees. This reinforces the need to develop rules and regulations aimed exclusively at determining the quality of honey from stingless bees. The growing demand for products from stingless bee also justifies additional studies and more complete approaches, due to the large number of species that are still poorly studied or that have not even been studied yet. Additional conservation treatments should be considered to increase the shelf life of honey, as well as to facilitate commercialization by informal producers. The chemical profile of native bee honeys has been little explored, which limits the quantification of classes of compounds, so more comprehensive studies regarding chemical characterization are needed. The advancement of scientific knowledge related to the particularities of honey of each species of stingless bee will be of fundamental importance in order to increase the value of its products, especially if it is conducted to identify and enhance regional aspects. This type of study has an urgent appeal in view of the current scenario in which the importance of conservation of the environment has been much questioned worldwide. As such, it will allow honey farmers to generate income effectively from the standing forest.

AUTHORS' CONTRIBUTIONS

ECAS and AF discussion of chemical data and wrote the paper and participated in the revisions of it; CM discussion of aspects of morphology, management, and conservation of stingless bees.

Miel d'abeille sans dard (Hyménoptères, Apidés, Méliponnes): Un examen du contrôle de la qualité, du profil chimique et du potentiel biologique.

abeilles sans dard / qualité du miel / composition chimique.

Honig von Stachellosen Bienen (Hymenoptera, Apidae, Meliponini): Ein Review über Qualitätskontrolle, chemisches Profil und biologisches Potential.

Stachellose Bienen / Honigqualität / chemische Zusammensetzung.

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Perfil químico, físico-químico, sensorial e atividade antioxidante do mel de *Scaptotrigona depilis* submetido a diferentes tratamentos.

Artigo 2

Perfil químico, físico-químico, sensorial e atividade antioxidante do mel de *Scaptotrigona depilis* submetido a diferentes tratamentos.

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Abstract: O mel de abelhas sem ferrão apresenta diferenças, comparado ao mel produzido por *Apis mellifera* e para sua adequação aos paramentros de qualidade estabelecido são previstos tratamentos pós-colheita. Nesse contexto, o objetivo deste estudo foi determinar o perfil químico, os parâmetros de qualidade, atividade antioxidante, além da avaliação sensorial o mel de *S. depilis* submetidos a diferentes processos de tratamento. Os méis tratados não sofreram alterações significativas no teor de fenólicos avaliados após 180 dias. Os parâmetros físico-químicos que apresentaram diferenças significativas foram o teor de acidez, umidade, atividade diastasica e cor para o mel maturado e o teor de HMF para os tratamentos desumidificado e pasteurizado. Os compostos voláteis majoritários se diferenciaram em cada tratamento em relação ao mel in natura. O perfil sensorial de aceitação mostrou diferenças significativas para alguns dos atributos avaliados e a preferência dos consumidores foi pelo mel in natura.

Keywords: Voláteis, headspace, qualidade, fenólicos.

Introdução

As abelhas sem ferrão compreendem um amplo grupo de abelhas eusociais, com aproximadamente 500 espécies descritas, das quais 61 gêneros estão distribuídos em diferentes partes do mundo, com maior ocorrência nas Américas de acordo com a cataloção de Pedro (2014). Dentro deste grupo, destaca-se o gênero Scaptotrigona que compreende 22 espécies descritas, distribuídas pela região neotropical (CAMARGO e PEDRO, 2007).

As estratégias de estoque do mel de *Apis mellifera* se diferenciam daquelas adotadas pelas abelhas sem ferrão. No caso das *Apis mellifera* as abelhas retiram a umidade até um determinado nível em que os microrganismos não conseguem mais se reproduzir e com isso pode ficar estocado por muitos anos sem deteriorar. Já o mel das abelhas sem ferrão após sua colheita continua sofrendo modificações físicas, químicas e organolépticas, gerando a necessidade de produzi-lo dentro de níveis elevados de qualidade e controlando todas as etapas do seu processamento (VIT et al 1994; SOUZA et al, 2006).

O entrave principal da comercialização do mel de meliponíneos é o teor expressivo de água que confere instabilidade ao produto ao longo do tempo, pois é muito suscetível a fermentação. Para superar os problemas decorrentes disso, são necessárias boas práticas de coleta, visando a redução da contaminação por microrganismos. Depois de coletado, alguns métodos de beneficiamento podem ser aplicados para auxiliar na conservação desse produto (CONTRERA et al., 2011; VENTURIERI et al., 2007).

Alguns estudos têm avaliado a estabilidade do mel, após o emprego desses tratamentos, e os resultados tem levado a discussão as possíveis alterações que o mel pode sofrer e, nesta perspectiva, o objetivo desse trabalho é avaliar perfil de voláteis, teor de fenólicos e atividade antioxidante, além dos parâmetros físico-químicos e análise sensorial do mel de *Scaptotrigana depilis* submetido a diferentes tratamentos após de 180 dias de estocagem.

Materiais e métodos

Coleta

Doze quilogramas (12 kg) de mel foram coletados de diversas colônias de *Scaptotrigona depilis* no meliponário da Embrapa Meio Ambiente, em Jaguariúna-SP, sob a coordenação do pesquisador Dr. Cristiano Menezes.

Processamento do mel

A amostra de mel coletado em diferentes colônias foi dividida em quatro porções. Uma parte do mel foi imediatamente submetida às análises de composição de voláteis e quantificação de fenólicos, físico-químicas e atividade antioxidante. As demais foram submetidas a diferentes tratamentos. Todos os processos descritos foram realizados em triplicata. **Refrigeração**: consistiu em manter o mel sob refrigeração em refrigerador doméstico armazendado em frasco de vidro transparente com tampa. **Desumidificação:** uma porção do mel (250g) foi transferida para refratários de vidro com formato retangular (20 x 30

cm), distribuído uniformemente formando uma camada fina (1 cm) e acondicionados em geladeira do tipo Frost Free, para auxiliar na desumidificação utilizou-se sílica gel. A umidade foi monitorada com um refratômetro até obtenção do Brix de 70% e então transferidas para frascos de vidro transparente fechado e mantido a temperatura ambiente protegido da luz e do calor. **Pasteurização:** Em garrafas de vidro transparente aberta, porções de mel (250 g) foram submetidas a aquecimento em banho-maria a 70°C por 15 segundos. Em seguida o mel foi submetido a refrigeração em banho de gelo por 1 hora e o frasco foi fechado. As garrafas foram mantidas a temperatura ambiente livres da iluminação direta. **Maturação:** Em uma garrafa de vidro transparente com tampão (algodão e gaze), o mel (250 g) foi mantido ao abrigo da luz e a temperatura ambiente. Todos os tratamentos foram realizados com a mesma amostra composta de mel em triplicatas para cada tratamento e foram mantidas nas condições descritas acima por um período de 180 dias.

Quantificação de fenólicos:

O teor de fenólicos foi determinado empregando o método espectrofométrico com reagente de Follin-Ciocalteu e leitor de placas (Synerg HT, BioTek) adaptando a metodologia descrita por Pontis et al (2014). Em placas de 96 poços, foram pipetadas alíquotas para curva de calibração, a cada poço foi transferido 20µL de Follin-Ciocalteu, 120 µL de solução aquosa de carbonato de sódio 5% (m/v) e 100 µL de soluções em concentração crescente de ácido gálico (0,001, 0,002, 0,003, 0,004 e 0,005 $mg.mL^{-1}$), para completar o volume adicionou-se 60 µL de água destilada. Para as amostras, 20 µL de cada solução aquosa dos méis (1g/mL) foram adicionados a 20µL de Follin-Ciocalteu e 120 µL de solução aquosa de carbonato de sódio 5%(m/v) e volume completado com 140 µL de água destilada. A placa ficou ao abrigo da luz por 2 horas, em seguida foram analisadas no comprimento de onda de 798 nm. As análises foram realizadas em triplicatas e o teor determinado por regressão linear.

Atividade antioxidante:

A atividade antioxidante foi determinada pelo método do sequestro do radical livre 2,2-difenilpicrilhidrazila utilizando um leitor de placas (Synerg HT, BioTek) de acordo com a metodologia descrita por Mensor et al (2001) com adaptações. Em placa de 96 poços foram pipetados 100µl das soluções de trolox $^{-1}$ nas concentrações 0,002, 0,004, 0,006, 0,008 e 0,01 $mg.mL^{-1}$, adicionou-se 150 µL de uma solução de DPPH a 1mM. A partir dessas alíquotas foi construída uma curva de calibração. Para as amostras, alíquotas de 100µL das soluções de mel *in natura* e de cada tratamento na concentração de $1g.mL^{-1}$ as quais foram adicionados 150µL

da solução de radical DPPH. A partir da equação da reta obtida da curva de calibração calculou-se a atividade antioxidante, expressa em mg de trolox/Kg de mel.

Parâmetros físico-químicos do mel:

As determinações foram feitas em cada amostra em triplicada, inicialmente no mel in natura pouco tempo após a colheita e após o período de 180 dias para as amostras submetidas aos tratamentos. Para açúcares redutores (CAC/Vol. III, Supl.2, 1990, 7.1), umidade (A.O.A.C.16th Edition, Rev.4th, 1998-969.38B), sacarose aparente (CAC/Vol. III, Supl.2, 1990, 7.2), Sólidos insolúveis em água (CAC/Vol. III, Supl.2, 1990, 7.4), Minerais (CAC/Vol. III, Supl.2, 1990, 7.5), Acidez (A.O.A.C.16th Edition, Rev.4th, 1998-962.19), atividade diastática (CAC/Vol. III, Supl.2, 1990, 7.7), hidroximetilfurfural (A.O.A.C.16th Edition, Rev.4th, 1998-980.23), Brix (A.O.A.C.16th Edition, Rev.4th, 1998-969.38B) e cor (Brasil. Ministério da Agricultura,1981).

Extração de voláteis por coleta dinâmica:

Para o sistema de extração de voláteis por coleta de dinâmica, utilizou-se uma corrente de ar de nitrogênio (N_2) a um fluxo de 1mL.min^{-1} e um agitador magnético. Em um balão de fundo redondo de duas bocas 100 mL, foram adicionados 50 g de mel dissolvido em 30 mL de solução de cloreto de sódio a 10%. Tubos de vidro (5 cm) empacotados com 50 mg do adsorvente Porapak-Q, foram conectados ao balão com auxílio de reduções de junta e mangueiras, o tubo conectado a entrada do gás, foi utilizado como branco e o outro concentrou os voláteis da amostra. Após 3 horas os voláteis adsorvidos foram extraídos com diclorometano bidestilado (1 mL) e concentrados com nitrogênio (N_2). A massa de voláteis obtida não foi determinada devido a alta volatilidade e baixa concentração das amostras obtidas.

Análises por cromatografia a gás acoplada a espectrometria de massas:

Um cromatógrafo a gás da marca Shimadzu (modelo GC-2010) acoplado a um espectrômetro de massa do mesmo fabricante (modelo QP2010 Plus) foi utilizado para a análise de compostos voláteis. A separação foi realizada usando uma coluna capilar de sílica fundida (RTX-5MS, 30 m × 0,25 mm × 0,25 μm). A temperatura do injetor era de 220° C, a temperatura da interface era de 280 ° C e a temperatura da coluna foi programada para aumentar de 35 °C a 3° C.min $^{-1}$ a 220°C, atingida essa temperatura o aumento gradual foi 20°C.min $^{-1}$ até 310°C. O hélio foi utilizado como gás de arraste a uma vazão constante de

1,02 mL min $^{-1}$. Os espectros de massas foram adquiridos na faixa de *m/z* 40-600 usando ionização eletrônica com um poder de ionização de 70 eV e a fonte de íons a 260 °C.

Determinação dos constituintes:

A composição dos voláteis foi determinada por comparação dos valores de seus índices de retenção obtidos a partir de uma série homóloga de *n*-alcanos (C_7-C_{30}) analisados nas mesmas condições, calculados de acordo com o método de Van den Dool e Kratz, além da comparação dos espectros de massas com os dados das bibliotecas digitais Wiley 8 e FFNSC 1.2, do banco de dados NIST e com dados da literatura existente (Adams, 2017, Pherobase).

Análise sensorial

Os méis tratados (refrigerado, pasteurizado, desumidificado e maturado), além da amostra do mel *in natura*, foram submetidos à análise sensorial por uma equipe de 20 provadores não treinados, recrutados após o preenchimento do formulário de entrevista online (https://forms.gle/XCGSuY4n3hYuEgRz7), os quais assinaram o termo de consentimento livre e esclarecido para participação da pesquisa, sendo aplicados testes de ordenação-preferência e aceitabilidade mediante escala hedônica. O teste de ordenação-preferência foi utilizado para determinar a preferência entre cada amostra. Neste estudo, não foram avaliadas diferenças entre os sabores. O julgador ordenou as amostras estabelecendo uma escala decrescente das amostras mais preferidas para as menos preferidas (MINIM, 2006). A escala hedônica estruturada de 9 pontos, desde desgostei muitíssimo (1) até gostei muitíssimo (9) avaliou os parâmetros de cor, aroma, sabor, acidez, e aparência global (CHAVES, 2001) do mel submetido a diferentes tratamentos.

Os provadores receberam aproximadamente 5 mL de cada amostra com temperatura de 25ºC, em colheres descartáveis, codificadas com números aleatórios de três dígitos (MININ, 2006). O estudo foi previamente submetido ao Comitê de Ética e Pesquisa por meio da plataforma Brasil e sua execução foi aprovada, conforme parecer 5.085.140.

RESULTADOS E DISCUSSÕES

Compostos fenólicos e atividade antioxidante

O mel de *S. depilis* submetido a diferentes tratamentos teve seu teor de fenólicos quantificado, em que os valores estão listados na Tabela 1, além dos valores da atividade antioxidante, determinada pelo método do sequestro do radical livre 2,2-difenilpicrilhidrazila.

A literatura descreve forte correlação entre a atividade antioxidante e o teor de fenólicos, e os resultados obtidos corroboram com essa correlação.

Tabela 1- Resultados da quantificação de fenólicos e atividade antioxidante no mel de *S. depilis* submetida a diferentes tratamentos.

	Fenólicos (mg GAEq.Kg^{-1})	**DPPH$^{\cdot}$ (mg TE. Kg^{-1})**
In natura	0.43±0.01 ^{ab}	2.37±0,12 ^{a}
Refrigerado	0.45±0,05 ^{ab}	2.33±0,11^{a}
Maturado	0.45±0.15 ^{ab}	2.16±0.08 ^{ab}
Pasteurizado	0.47±0.015 ^{a}	2.26±0.07 ^{ab}
Desumidificado	0.39±0.02 ^{b}	2.07±0.04 ^{b}

Legenda: Resultados expressos como média ± desvio padrão. Médias com letras iguais na mesma coluna não diferem significativamente de acordo com o teste de Tukey a $p \leq 0,05$.

Houveram variações discretas no teor de fenólicos nos méis submetidos à tratamentos em relação ao mel in natura, demonstrando que em todos houve preservação destas importantes moléculas naturais, ou seja, os tratamentos não afetam o teor desses compostos ao longo do tempo avaliado. Nas mínimas alterações percebidas, o mel desumidificado é o que apresentou o menor teor desses compostos e, consequentemente, exibiu menor potencial antioxidante. Para os demais tratamentos, não houve diferenças significativas na atividade antioxidante.

Estudos relacionados à determinação do teor de fenólicos, bem como o potencial antioxidante do mel de abelhas sem ferrão após tratamento e por determinado período de tempo não foram encontrados na literatura consultada, o que evidencia a importância do estudo para avaliação da preservação desses compostos após o beneficiamento do mel e sua estocagem. Pelo dados obtidos pode-se dizer que os tratamentos pouco alteraram a atividade antioxidante, o que é um dado muito bom.

Parâmetros físico-químicos

Os parâmetros físico-químicos (açúcares redutores, acidez livre, atividade diastásica, cinzas, sólidos insolúveis, sacarose, HMF, brix e umidade) do mel submetidos aos tratamentos de pasteurização, desumidificação, refrigeração e maturação, além do mel in natura estão listados na Tabela 2 .

Tabela 2- Resultados dos parâmetros físico-químicos do mel *in natura* de *S. depilis* e submetido a diferentes tratamentos

	Açúcares redutores	Acidez livre	Atividade diastásica	Minerais (cinzas)	Sólidos insolúveis	Sacarose	HMF	Brix	Umidade	Cor
In natura	66,65±0,13^{a}	29,39±0,11^{b}	9,71±0,23^{a}	0,42±0,04^{b}	0,06±0,008^{a}	1,41±0,46^{a}	1,20±0,61^{b}	74,05±0,041^{a}	24,35±0,01^{b}	Âmbar
Refrigerado	68,97±1,98^{a}	26,26±0,56^{b}	8,90±0,31^{a}	0,74±0,07^{a}	0,08±0,01^{a}	0,71±0,17^{a}	1,02±0,45^{b}	74,08±0,03^{a}	23,98±0,04^{b}	Âmbar
Maturado	66,85±0,63^{a}	117,02±6,06^{a}	1,70±0,74^{b}	0,74±0,05^{a}	0,06±0,01^{a}	0,43±0,0001^{a}	1,61±0,74^{b}	70,03±0,34^{b}	28,26±0,29^{a}	Âmbar escuro
Pasteurizado	67,61±2,98^{a}	24,21±1,38^{b}	7,57±0,87^{a}	0,48±0,04^{ab}	0,06±0,0001^{a}	0,58±0,17^{a}	10,17±0,70^{a}	74,79±0,01^{a}	23,58±0,02^{b}	Âmbar
Desumidificado	71,34±0,63^{a}	25,95±2,94^{b}	5,13±3,29 ^{ab}	0,56±0,15^{ab}	0,06±0,01^{a}	0,49±0,01^{a}	10,81±0,28^{a}	78,30±0,43^{a}	20,06±0,43^{c}	Âmbar

Legenda: Resultados expressos como média ± desvio padrão. Médias com letras iguais na mesma coluna não diferem significativamente de acordo com o teste de Tukey a $p \leq 0,05$.

O emprego de tratamentos no mel de abelhas sem ferrão tem como objetivo conferir maior segurança alimentar, impedindo a proliferação de microrganismos, tornando-o mais estável ao longo do tempo, nesse sentido, avaliando os parâmetros de qualidade do mel *in natura* e após tratamentos observa-se que o índice de acidez no mel maturado é significativamente maior que no mel in natura, enquanto que os demais tratamentos exibiram valores inferiores ao mel não tratado. A atividade diastásica do mel maturado foi a menor comparada às demais, e seu teor de umidade é o mais elevado, além da cor, que se difere das outras amostras. A pasteurização e desumidificação proporcionou um mel menos ácido, mas com teores HMF elevados.

Em comparação com os resultados obtidos por Menezes et al. (2018) que empregou o tratamento de pasteurização com mel de *M. fasciculata* e *M. flavolineata*, os resultados mostraram que o processo influenciou significativamente o teor de HMF (9.43±0.09 e 43.10±0.85, respectivamente). Além disso, umidade, sacarose aparente também sofreram mudanças significativas quando comparadas com o mel não pasteurizado, diferente dos resultados encontrados para o mel pasteurizado de *S. dellis* que não apresentou mudanças significativas para esses últimos parâmetros. Os valores alterados de HMF podem apontar alterações importantes geradas por armazenamento prolongado em temperatura ambiente alta e/ou superaquecimento, de acordo com o Fallico et al. (2004), a formação de HMF ocorre devido a desidratação de hexose catalisada por ácidos, aliada às propriedades químicas do mel.

Em relação ao mel desumidificado, os estudos de Alves et al. (2012) mostraram que este tratamento conferiu boa estabilidade para os parâmetros umidade, açúcares redutores, sacarose aparente, acidez e HMF durante um período de armazenamento de 180 dias do mel de *Tetragonisca angustula*. Com exceção do teor de HMF e umidade, os demais parâmetros para o mel desumificado de *S. depilis* ao longo do mesmo período não apresentaram diferenças significativas quando comparados ao mel in natura.

O mel de Tiúba, maturado a 30°C, não provocou mudanças significativa nos parametros físico-químico, seu teor de acidez apresentou um leve aumento, de 23.87±1,21 para 26.10±1.20, quando comparado ao mel não tratado, diferente do observado para o mel de *S. depilis* maturado que teve seu teor elevado significativamente, de 29.39±0.11 para 117.02±6.06.

Voláteis do Mel

Foram identificados 42 compostos voláteis no mel de *S. depilis*, equivalente a mais de 85% dos constituintes detectados, em que seus teores variaram entre as amostras tratadas após o período de 180 dias. A Tabela 3 reúne os compostos identificados nos diferentes tratamentos.

Tabela 3-Compostos voláteis extraídos por headspace dinâmico do mel de *S. depilis* submetido a diferentes tratamentos.

	Compounds	**TR**	**IR_C**	**IR_L**	**Natura**	**Maturado**	**Refrigerado**	**Desumidificado**	**Pasteurizado**
1	2-Hydroxy-3-pentanone [b]	6.051	828	821					0,29±0,15
2	Ethyl 2-hydroxypropanoate[b]	6283	833	836	4.12±0.04	3,00±0,25			
3	Heptane-2,3-dione[b,c]	6925	847	-	0.47±0.03	0,59±0,01	0,92±0,11	0,57±0,02	0,42±0,03
4	Heptan-4-one[b,c]	7.358	857	860	3.45±0.13		3,28±0,19	3,34±0,08	2,89±0,01
5	Ethyl isovalerate [b]	7508	861	858	1.89±0.16	0,72±0,06			
6	2-Methyl hexan-3-ol, [b]	7692	865	858	2,88±0.21		3,98±0,23	5,84±0,06	4,78±0,11
7	3-Methyl butanoic acid,[b,c]	7850	868	875	0.26±0.02	0,18±0,07			
8	Hexan-1-ol [b,c]	8.050	873	867		0,19±0,01	0,15±0,01		0,14±0,02
9	Heptan-2-one [b,c]	8.858	891	889			0,33±0,02		0,09±0,00
10	Heptan-4-ol [b,c]	8.950	893	889				0,08±0,00	
11	Heptan-2-ol [b,c]	9.217	899	901		0,63±0,03		0,10±0,05	0,30±0,10
12	2-Butoxy ethanol, [b,c]	9.425	904	904			0,31±0,09		
13	2,6-Dimethyl heptan-4-one [b,c]	11.042	940	943		26,16±0,81	41,83±0,28	33,12±0,52	
14	2-Methyl Heptan-3-one [b,c]	11125	942	938	28.09±0.57				34,20±0,20
15	2,7-Dimethyl octan-4,5-diol [b,c]	11.192	943	944	7.34±0.39	2,65±0,17	28,43±0,51	34,72±0,08	31,68±0,13
16	Benzaldehyde [a]	11708	955	960	0.55±0.39			0,47±0,25	
17	2-hydroxy-3-methyl Butanoic acid ethyl ester [b,c]	12.000	962	968	2.55±0.03	0,39±0,04			
18	Mesitylene [c]	13133	987	995	0.65±0.02				
19	Benzyl alcohol [a,b]	15117	1029	1026	0.95±0.09				
20	2,6-Dimethylheptan-4-ol [b,c]	15.183	1031			2,68±0,19			
21	Ethyl 2-hydroxycaproate [b,c]	16258	1053	1061	1.02±0.02				
22	*cis* Linalool oxide [a]	16892	1066	1072	2.92±0.18	3,04±0,03	0,51±0,04	0,31±0,01	1,00±0,01
23	tetramethyl Pyrazine [b,c]	17542	1080	1086	0.34±0.02	0,27±0,04	0,45±0,07	0,53±0,08	0,32±0,01
24	*trans* Linalool oxide [a]	17633	1082	1086	1.23±0.06	1,12±0,08	0,51±0,05	0,60±0,05	0,51±0,01
25	Linalool[a]	18225	1094	1096	0.49±0.10	1,45±0,20	4,41±0,09	0,36±0,01	0,82±0,01
26	Hotrienol[a,b,c]	18492	1100	1103	10.09±0.09	36,94±0,29	1,09±0,08	0,44±0,02	13,04±0,08

27	1,3-dioxolane-2-methanol, 2,4-dimethyl- [c]	18.717	1105	-				0,58±0,15	
28	Phenethyl alcohol [b,c]	18858	1108	1108	3.66±0.2	5,42±0,11	1,53±0,06	7,65±0,19	0,80±0,01
29	Isophorone [b,c]	19133	1113	1121	0.35±0.04	0,35±0,03	0,15±0,04	0,13±0,03	0,28±0,01
30	Isophorone <4-keto-> [b]	20250	1137	1145	0.69±0.05		1,49±0,03	0,82±0,07	1,58±0,02
31	Lilac aldehyde B [b,c]	20.617	1145	1154				0,25±0,02	
32	Isoneroloxide[b,c]	20.817	1149	1147		0,45±0,02			
33	Dihydrooxophorone[b,c]	21.392	1161	1170		0,43±0,03	0,43±0,06	0,09±0,01	0,14±0,01
34	Ethyl benzoate [b,c]	21567	1165	1169	3.16±0.07	0,36±0,04			
35	nonan-1-ol [a]	21650	1166	1169	0.31±0.05	0,27±0,02	0,26±0,05	0,12±0,01	0,25±0,06
36	3,7-dimethylocta-1,5-dien-3,7-diol [b,c]	22.483	1184	1176		0,86±0,05			
37	Hex-3(Z)-enyl butyrate [b,c]	22508	1185	1186	0.7±0.04		0,69±0,01	0,12±0,02	0,25±0,02
38	Verbenone [a,b]	23.758	1211	1205					0,45±0,02
39	Phenylacetate <ethyl-> [b,c]	25100	1240	1246	10.50±0.29	4,02±0,02			
40	Acetic acid, 2-phenylethyl ester	25.583	1251	1256		0,27±0,08			
41	Butanone <3-hydroxy-4-phenyl-2> [b,c]	29467	1337	1342	0.33±0.02		1,36±0,04	0,50±0,05	0,99±0,06
42	Benzenepropanoic acid, ethyl ester [b,c]	29675	1342	1348	0.39±0.01				
	Percentual de compostos identificados				88.87	92.59	92.12	90.72	95.35

Legenda: IR_C: Índice de Retenção Calculado; IR_L: Índice de Retenção da literatura; [a]Identificação pela comparação com Adams, 2017; [b] Identificação por comparação com a biblioteca do NIST; [c]Identificação pela iblioteca Willey ou FFNSC.

O mel na forma *in natura* apresentou como constituintes principais o 2-methyl heptan-3-one (28.09±0.57%), ethyl phenylacetate (10.50±0.29%), hotrienol (10.09±0.09%) e 2,7-dimethyl-4,5-octandiol (7.34±0.39 %), enquanto que no mel maturado foram predominantes o hotrienol (36,94±0,29%), 4-heptanone, 2,6-dimethyl- (26,16±0,81%), e phenethyl alcohol (5,42±0,11 %). O mel refrigerado tem como compostos majoritários o 2,6-dimethyl heptan-4-one, (41,83±0,28%), 2,7-dimethyl-octan-4,5-diol (28,43±0,51%) e o linalool (4,41±0,09%), no desumidificado também predominam o 2,7-dimethyloctan-4,5-diol (34,72±0,08) e 2,6-dimethyl heptan-4-one, (33,12±0,52), além do phenethyl alcohol (7,65±0,19%). Os voláteis do mel pasteurizado foram os mesmos do mel in natura com diferenças significativas no teor, o 2-methyl heptan-3-one (34,20±0,20%), 2,7-dimethyl octan-4,5-diol (31,68±0,13%) e hotrienol (13,04±0,08%).

De modo geral quando comparadas as composições dos méis submetidos a tratamento com o mel *in natura*, observa-se que 16 compostos não estão presentes no mel *in natura*, outros tiveram aumento significativo em sua concentração, a exemplo disto, temos o hotrienol, que

apresentou um aumento considerável na amostra maturada, mas que na amostra desumidificada diminuiu significativamente seu teor. A presença desse composto em méis de *Apis mellifera* foi associada a diferentes origens botânicas, sendo relatado como típico do mel cítrico, nos quais foi encontrado em alta proporção (Alissandrakis et al., 2007; 2009). Entretanto, outros estudos mostram que esse composto pode ser produto de degradação do mel, sendo produzido por variações térmicas. De acordo com Jercokovic et al (2009;2010;2014) o hotrienol é produto da desidratação do 3,7-dimethylocta-1,5-diene-3,7-diol (terpenediol I), em que as condições quentes e ácidas da colmeia podem promover a desidratação do diol precursor do mesmo. Nessa perspectiva, esperar-se-ia que a concentração do hotrienol na amostra pasteurizada fosse mais elevada, tendo em vista que neste tratamento o mel é submetido a aquecimento. O aumento considerável do hotrienol no mel maturado sugere que o processo de fermentação do mel foi o fator determinante na produção deste composto.

O ethylphenylacetate um dos constituintes predominantes no mel *in natura*, só foi detectado no mel maturado, mas com menor teor, este composto é descrito como odor associativo ao mel, está presente em cervejas com sabor de mel, além disso, foi detectado em altas concentrações entre os voláteis de amostras de vinhos, sendo responsável pelo sabor e odor característicos de mel (Campo et al, 2012).

Os resultados apontam que os méis submetidos a diferentes tratamentos, avaliados após 180 dias apresentam composição que se difere do mel *in natura*, em que são observados compostos exclusivos principalmente no mel desumidificado, no qual foram identificados 4 compostos presentes apenas nesta amostra. No mel maturado foram detectados exclusivamente 3 compostos, enquanto que nos méis refrigerado e pasteurizado esse quantitativo foi apenas de 1 composto.

Este é o primeiro relato da composição dos voláteis do mel de *S. depillis* submetidos aos tratamentos de pasteurização, desumidificação, refrigeração e maturação, as diferenças observadas são uma importante contribuição ao conhecimento da composição do mel desta abelha e mostram a necessidade do aprofundamento dos estudos para justificar a ocorrência de determinados compostos nos diferentes tratamentos. Por ser uma matriz complexa o mel oferece inúmeras possibilidades para origem de compostos, tendo em vista que apresenta uma microbiota capaz de promover diferentes tipos de rações, além disso, as condições de estocagem, bem como sua duração também são fatores que influenciam.

Analise Sensorial

Na Tabela 3 está apresentado o resultado do teste de ordenação usado para avaliar a preferência de cinco tratamentos aplicados a méis de *S. depilis* apresentados a 20 julgadores. Os resultados do teste de Friedman mostraram que os valores seguidos da mesma letra não diferem entre si a de 5% de significância.

Tabela 3 - Avaliação sensorial de preferência do mel de *S. depilis* por ordenação para as amostras tratadas

		Mel				
		DES	**PAS**	**IN**	**REF**	**MAT**
Soma de ordens		60b	58b	44b	63b	76a
Diferença vs.	DES	-	2^{ns}	16^{ns}	3^{ns}	16^{ns}
	PAS	-	-	14^{ns}	5^{ns}	18^{ns}
	IN	-	-	-	19^{ns}	32^{*}
	REF	-	-	-	-	13^{ns}

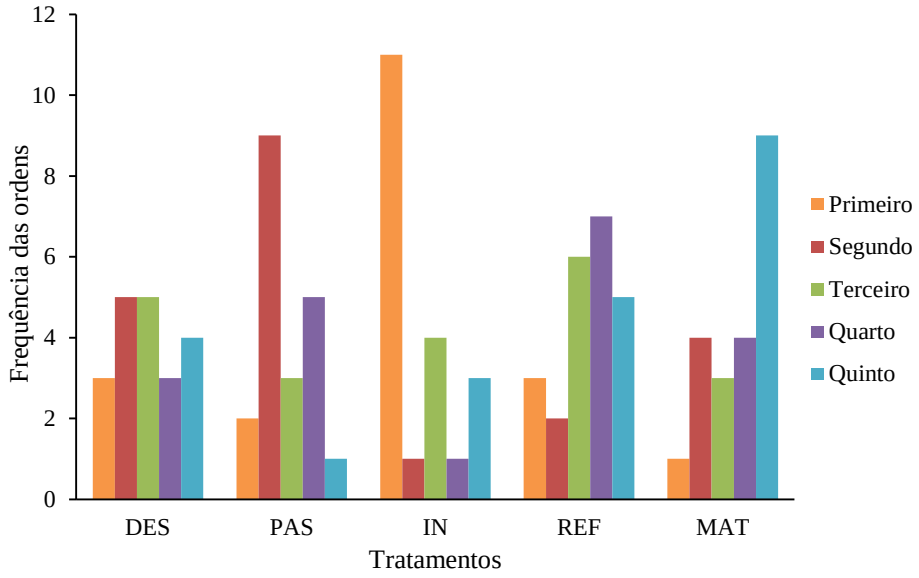
ns = não significativo. Valor absoluto crítico de diferença mínima significativa (dms) α = 28 (NEWELL; MACFARLANE, 1987.

Nas amostras avaliadas, os módulos da diferença foram inferiores a DMS = 28 (diferença mínima significativa) nos tratamentos desumidificado (DES), pasteurizado (PAS), *in* natura (IN) e refrigerado (REF), sendo estas as amostras preferidas em detrimento do mel submetido ao processo de maturação (MAT). No teste de ordenação as menores somas indicam as formulações mais preferidas, enquanto as maiores somas indicam menor preferência, nesse sentido, mel *in natura* e o mel pasteurizado seriam as amostras preferidas, porém não houve diferença significativa a 5% no Teste de Friedman, que permitisse estabelecer esta classificação.

Em relação ao mel maturado, este foi ordenado significativamente como a amostra menos preterida pelos provadores, sendo, portanto, um tipo de processamento menos aceitável pelo consumidor. Entretanto, análises complementares de outras atividades biológicas ou o emprego em outros segmentos como em comésticos, culinária ou farmacêutica podem ser considerados.

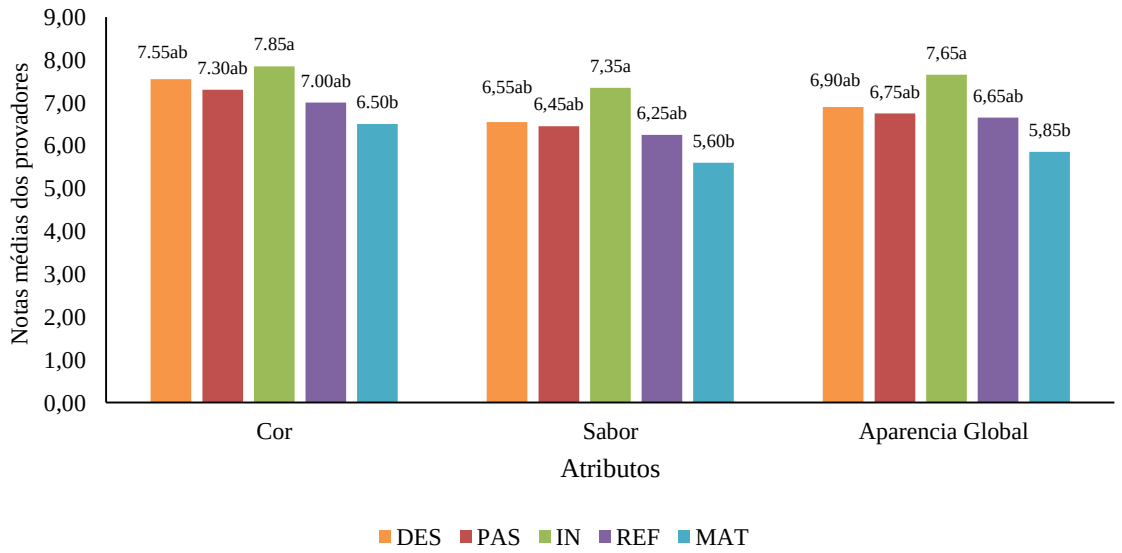
Entre os méis DES, PAS, IN e REF, não houve diferença significativa a 5% que classifique apenas uma amostra como preferida, entretanto, quando se observou o gráfico de frequência das ordens, verificou-se que a amostra (IN) ficou mais vezes em primeiro lugar, seguida pelas amostras DES e REF e por último a amostra PAS (**Figura 1**).

Figura 1- Resultados do teste de ordenação-preferencia do mel de *S. depilis* submetido a diferentes tratamentos.



As aceitabilidades avaliadas por meio da escala hedônica mostraram diferença significativa para os atributos de cor, sabor e aparência global. Já nos atributos aroma e acidez, não foi observada diferença suficiente que gerasse alteração na percepção dos provadores (Figura 2), mesmo o mel maturado sendo mais ácido que as demais amostras e o perfil de voláteis extraídos dos méis tratados sendo diferente quimicamente.

Figura 2- Médias dos atributos que apresentaram variações significativas entre os tratamentos.



As médias das notas dos atributos cor, aroma e aparência global apresentaram padrões de preferências semelhantes, nos quais o mel in natura foi classificado como “gostei muito” na escala estruturada de 9 pontos. As médias dos tratamentos desumidificado, pasteurizado e refrigerado não se diferenciação significativa do mel in natura. As menores médias foram relacionadas ao mel maturado, o que pode ser relacionado com o maior índice de acidez, aroma mais intenso e coloração mais escura, constata pelas análises físico-químicas e composição de voláteis.

A análise sensorial aplicada por Pires et al (2020) para o mel de duas espécies de abelhas sem ferrão, *Scaptotrigona* sp.(canudo amarela) e *Melipona interrupta* (jandaíra), apontaram para uma maior aceitação do mel de jandaíra submetido a refrigeração e pasteurização. Para de Scaptorigona p. a preferência foi pelo mel pasteurizado com correlação positiva com o aroma.

CONCLUSÃO

O teor de fenólicos não sofreu alteração em decorrência dos tratamentos empregados ao mel. Os parâmetros físico-químicos diferenciaram-se significativamente no mel maturado em relação ao nível de acidez, umidade, atividade diastásica e cor, para o mel pasteurizado e desumidificado a diferença foi mais evidente para o teor de HMF. Em relação ao perfil de voláteis, o estudo revela a importância de um maior aprofundamento na determinação da origem dos constituintes, mas já apresenta resultados relevantes em relação à interferência dos tratamentos na composição do aroma do mel. O perfil sensorial aponta para uma boa aceitação das amostras, e que os atributos avaliados não foram afetados pelos tratamentos, no entanto, a preferência dos julgadores foi pelo mel in natura.

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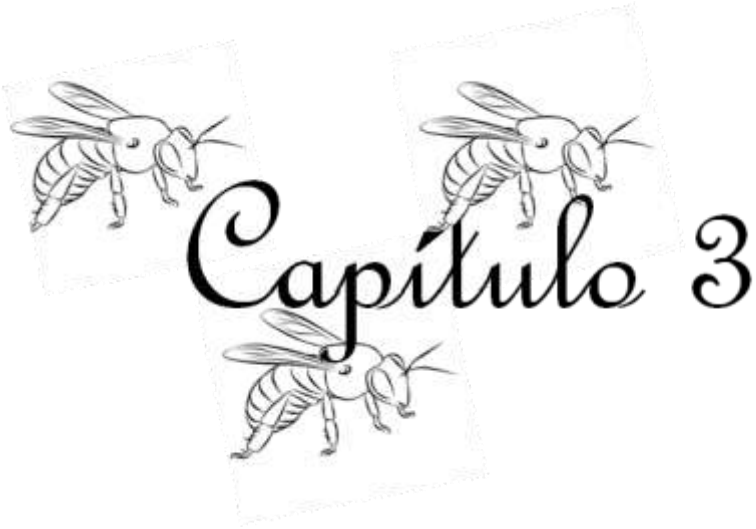

Propolis de abelhas sem ferrão: Breve revisão da composição química, perfil físico-químico e potencial biológico.

INTRODUÇÃO

Própolis é um produto elaborado a partir de resinas coletadas por abelhas em brotos e exsudados de plantas, com a finalidade de proteger as colmeias reparando frestas ou danos, impedindo a invasão de predadores e mantendo a temperatura interna. Sua composição é bastante variada em decorrência de inúmeros fatores, e a importância de seu estudo está nessa diversidade. Além disso, do ponto de vista ecológico estudar a própolis e não só a resina, ainda que estas componham aquelas, fornece informações da relação inseto-planta.

É um produto que tem sido muito investigado em relação à composição química e propriedades biológicas, sendo que a grande maioria dos estudos foram desenvolvidos com a própolis produzida por *Apis mellifera*, espécie com maior potencial produtivo, cujos produtos são comercializados. Os resultados obtidos com as amostras dessa espécie têm mostrado uma grande diversidade química diretamente relacionada a fonte vegetal, além de ter comprovada ação biológica. Nesse contexto a própolis produzida por outras espécies de abelhas, como no caso dos meliponíneos, apesar de ser pouco explorada, pode também ser fonte alternativas de compostos bioativos.

Pesquisas têm sido realizadas comparando-se os produtos de abelhas melíferas e meliponíneos, ambos coletados de uma mesma localização. Os dados obtidos nestes estudos muitas vezes mostraram que os recursos utilizados por *Apis mellifera* e pelas abelhas sem ferrão não são os mesmos, levando a diferentes atividades biológicas exercidas pelo mel e própolis produzidas por elas.

No Brasil, o estudo com própolis de *A. mellifera* de diferentes regiões conduziu a classificação em diferentes grupos. Park, Ikegaki e Alencar (2000), estudaram amostras oriundas de três regiões e a partir das características físico-químicas e propriedades biológicas classificaram as própolis em 12 grupos, sendo os cinco primeiros grupos são de amostras da região sul, as seis amostras do nordeste originaram os grupos de 6-11 e uma da região sudeste o grupo 12. Daugsch et al. (2006) com as própolis de região nordeste, encontraram características distintas para os grupos supracitados, principalmente a coloração, estas ficaram conhecidas como própolis vermelha que foram agrupados em um novo grupo de própolis. Vale ressaltar que as própolis originárias da região amazônica não foram estudadas, logo, não integram nenhum desses grupos.

PROPOLIS

Na arquitetura de uma colmeia, as abelhas utilizam diferentes materiais, alguns encontrados na natureza (resinas) e outros secretados pela própria abelha (cera). A própolis, utilizada na construção dos ninhos é constituída basicamente por resinas vegetais coletadas pelas abelhas de plantas lenhosas feridas e de flores. As resinas coletadas são trabalhadas pelas abelhas nos seus ninhos, seja numa forma pura (própolis pura) ou misturada com um pouco de cera (própolis mista ou cerume), algumas espécies de abelhas também adicionam à resina, terra ou barro, dando origem a geoprópolis. Essas substâncias têm como finalidade a proteção da colmeia reparando frestas ou danos, impedindo a invasão de predadores e mantendo a temperatura interna (NOGUEIRA-NETO, 1997).

A utilização da própolis na medicina popular é milenar, sua composição complexa confere inúmeras propriedades terapêuticas, o consumo tem sido cada vez mais difundido, amparado por pesquisas científicas que comprovam seus efeitos benéficos. Sua origem é diversificada, em cada região podem ser encontrados diferentes tipos de própolis com características próprias, definidas principalmente, pelo tipo de vegetação. Em virtude disso, muitos estudos têm sido conduzidos para elucidar a composição química e a atividade biológica da própolis em diferentes regiões do mundo (MIGUEL e ANTUNES, 2011).

A produção da própolis por abelhas sem ferrão ainda tem sido pouco explorada, isso se deve à carência de informações sobre as espécies, técnicas de colheita e formas de processamento desse material. Para dirimir essas situações, estudos voltados para análises do potencial produtivo, caracterização química e atividades biológicas estão sendo considerados.

PERFIL FÍSICO-QUÍMICO DA PRÓPOLIS DE ABELHAS SEM FERRÃO

No Brasil o comércio de produtos derivados de própolis com fins terapêuticos obedece a critérios regidos por legislação que preconiza parâmetros para o controle de qualidade (Brasil, 2001). Nesse dispositivo a própolis está inserida no contexto de produtos apícolas, por já ser beneficiada de apiários.

Com a própolis de abelhas sem ferrão a determinação do perfil físico-químico é pouco abordada, os estudos buscam principalmente a caracterização química e investigação de ação biológica. A falta de determinação de parâmetros de qualidade e identidade de própolis de abelha sem ferrão reflete a incipiência do uso do produto formalmente, o que limita a exploração.

Araújo et al. (2016) determinaram o teor de umidade, perda por dessecação, cinzas e cera da própolis das abelhas *Melipona scutellari* e *Melipona fasciculata*, que comparada aos obtidos

para a *A. mellifera*, apresentam valores inferiores para todos os parâmetros citados. Os resultados, entretanto, enquadram-se aos estabelecidos pela legislação. O teor de fenólicos exibido pelas própolis dessas abelhas foi significativamente maior e, consequentemente, apresentou melhor desempenho na inibição de radicais livres.

Lorini et al. (2018) determinaram alguns parâmetros físico-químicos das própolis produzidas por *Scaptotrigona polysticta* e *A. mellifera*, sob a ótica comparativa, o extrato da abelha sem ferrão apresentou maior teor de cinzas e menores teores de extrato seco, fenólicos e flavonoides totais. Não obstante, todos os extratos apresentaram valores dentro dos estabelecidos pela legislação.

Apesar dos valores obtidos para alguns parâmetros físico-químicos da própolis produzida por abelhas sem ferrão serem diferentes da própolis de *Apis mellifera*, obedecem ao estipulado pela legislação. Entretanto, mais estudos precisam ser realizados para melhor avaliação, considerando os fatores que influenciam na composição do material.

COMPOSIÇÃO QUÍMICA E ATIVIDADE BIOLÓGICA DE DA PRÓPOLIS ABELHAS SEM FERRÃO

Por ser oriunda de diferentes resinas vegetais a composição da própolis é bastante complexa, compostas não só por constituintes fixos, como também voláteis, existe ainda a possibilidade da alteração estrutural de moléculas por meio das enzimas salivares, adicionadas na produção desse produto (VANHAELEN e VANHAELEN-FASTRE, 1979). Desta forma, a caracterização da própolis em vários lugares no mundo é crescente, até mesmo com aquelas produzidas por abelhas sem ferrão, onde estudos destinados a caracterização química e atividades biológicas estão compilados no Quadro 1.

Quadro 1-Compostos detectados em própolis de meliponíneos

Espécie	Classe de compostos	Atividade biológica	Referências
Melipona compressipes	Ácidos alifáticos e aromaticos, álcoois, aldeídos e cetonas, diterpenos e triterpenos	-	BANKOVA et al. (1998)
Tetragona clavipes			
Melipona quadrifasciata			
Melipona compressipes	Ácidos, ésteres, álcoois, fenóis, aldeídos, monoterpenos, sesquiterpenos, hidrocarbonetos alifáticos e aromáticos	Antimicrobiana	BANKOVA et al. (1999)
Tetragona clavipes			
Melipona quadrifasciata			
Melipona quadrifasciata	ácidos, diterpenos e triterpenos	Antimicrobiana e citotoxica	VELIKOVA et al. (2000)
Tetragonisca angustula	Ácidos fenólicos	Antibacteriana	MIORIN et al. (2003)
Tetragonisca angustula	Àcidos, álcoois e triterpenos	Antimicrobiana	DOS SANTOS

			PEREIRA et. al. (2003)
Melipona beecheii	Mono e sesquiterpenos, alcanos, ácidos graxos e diterpenos	-	PINO et al. (2006)
Melipona fasciculata	detecção de fenólicos, triterpenos e saponinas por CCD e quantificação de flavonoides	-	DUTRA et al. (2008)
Trigona spinipes	Flavonoides e triterpenos	-	FREITAS et al. (2008)
Melipona fasciculata	Flavonoides, polifenois	-	CUNHA et al. (2009)
Scaptotrigona aff. postica	Detecção de terpenos e cumarias	Antitumoral	ARAÚJO et. al. 2011
Melipona subnitida	Fenilpropanoides e flavonoides	Antioxidante	SOUZA (2012)
Melipona interrupta	Flavonoides	Antioxidante	SILVA et al. (2013)
Scaptotrigona postica	Flavonoides glicosilados	Antiviral	COELHO et al. (2015)
Melipona scutellari	Fenólicos	Antioxidante	ARAÚJO et al. (2016)
Melipona fasciculata			
Scaptotrigona postica	Flavonoides	Antioxidante	FERREIRA et al., (2017)
Scaptotrigona depilis	Triterpenos na fração apolar e ácidos fenólicos e flavonoides na fração polar	Antioxidante, Citotóxica e tóxica	BONAMIGO et al., (2017)
Melipona quadrifasciata anthidioides			
Melipona quadrifasciata quadrifasciata	Ácidos fenólicos e flavonoides	Antioxidante e Antimicrobiana	TORRES et al. (2018)
Tetragonisca angustula			
Scaptotrigona polysticta	Ácidos fenólicos	Antifúngica	LORINI et al. (2018)
Frieseomelitta longipes	Benzofenonas preniladas e mono e sesquiterpenos	Antioxidante e antimicrobiana	SOUZA et al. (2018)

A complexidade na composição da própolis é atribuída a fatores como, vegetação, estação do ano e genética da abelha. E, estudos destinados a avaliação destes fatores são bem consolidados para a própolis produzida por *A. mellifera* (CASTRO et al., 2007; NUNES et al., 2009). Esses estudos subvencionam a busca por características específicas no perfil químico e atividade biológica da própolis em determinado local e época do ano, orientando manejo e coleta para produção de ototerápico.

Na maioria dos estudos a avaliação da atividade biológica foi realizada, sendo a antimicrobiana a principal delas. A própolis representa um mecanismo de defesa das abelhas contra microrganismos, já que na colmeia existem fontes ricas para sua proliferação como os açúcares presentes no mel, o que justifica seu bom desempenho na inibição destes. A atividade pode ser potencializada pela presença de compostos voláteis, pois seria útil que a ação não se dê somente pelo contato, mas também pelo ar, desta forma, as própolis com porções maiores de resina

que contenham significativa concentração de óleos essenciais podem exibir atividade de inibição consideravelmente maior.

Em relação a comparação de perfil químico de própolis de abelhas de diferentes espécies, reporta-se aos estudos realizados com própolis de *A. mellifera* e *Melipona beecheii*, originárias de Yucatán, México, nas quais, identificaram além de mono e sesquiterpenos, também alcanos, ácidos graxos e diterpenos. Muitos dos constituintes identificados estavam presentes em ambas as amostras com variações no teor (PINO et al.,2006). Esses resultados remetem a diferenças nos hábitos das abelhas, apesar de, segundo os autores, a composição vegetal ser semelhante nas áreas onde foram realizadas as coletas.

Souza et al. (2018) relatam a diferença de perfil químicos e propriedades biológicas tanto de voláteis, como de compostos fixos nas própolis de *A. mellifera* e *Frieseomelitta longipes* criadas em um mesmo ambiente, o que sugere que as própolis são compostas por resinas vegetais distintas ou por porções diferenciadas. Estudos que subsidiam esses fatos, indicam que as resinas de algumas espécies arbóreas não atraem abelha, embora estas plantas produzissem altas quantidades de resina e estarem próximas a árvores onde as abelhas foram coletadas (Leonhardt e Bluthgen, 2009; Leonhardt et al., 2011). Leonhardt et al (2010) relata ainda que as abelhas sem ferrão de Bornéu usam pistas olfativas para encontrar árvores para coletar resinas, as abelhas usam mono e sesquiterpenos para localizar e reconhecer a fonte de resinas.

ESPECTROMETRIA DE MASSAS NA CARACTERIZAÇÃO DO PERFIL QUÍMICO DE PRÓPOLIS

A espectrometria de massas tem sido uma importante aliada na elucidação da composição da própolis, em que estudos pioneiros já a utilizavam para determinar o perfil químico de amostras de própolis.

Sua versatilidade tem permitido acoplar a cromatografia gasosa e líquida, além da autonomia da análise por inserção direta. Entretanto, as análises por essa técnica proporcionam grandes volumes de dados que podem ser caracterizados de acordo com experimentos sequenciais onde são gerados espectros de fragmentação de íons precursores. Nesse contexto, para análises dos dados, é necessário o emprego de softwares automatizados que possam identificar compostos a partir de dados brutos. Diante disso, para transpor esses obstáculos, foi criada a plataforma Global Natural Products Social Molecular Networking (GNPS) (WANG et al., 2016; OLIVON et al., 2017) que viabiliza análises automáticas de espectrometria de massas e compartilhamento comunitário de espectros para bibliotecas espectrais colaborativas.

O molecular networking (MN) gerado a partir dos dados de MS/MS é baseado em gráficos que visam à organização de abundantes conjuntos de dados de espectrometria de massas, a partir da semelhança espectral entre os padrões de fragmentação de íons precursores diferentes, mas estruturalmente correlacionados. O agrupamento é visualizado em uma rede molecular, de modo que espectros de íons precursores de mesma *m/z* e que possuem espectros de fragmentação semelhantes são incorporados em um único espectro de consenso representado em um node, marcado pela massa original (*m/z*) dos íons precursores.

Essa técnica de processamento de dados foi adotada por Silva-Júnior et al. (2021) para análise de diferentes materiais coletados no ninho de *Scaptotrigona depilis*, dos quais foram identificados predominantemente flavonoides. O mapa químico gerado apresentou correlações entre os extratos da planta e dos materiais da abelha, em uma tentativa de investigar a simbiose existente entre o inseto e a planta.

PERSPECTIVAS FUTURAS

Para a efetiva introdução da própolis no mercado, a padronização de coletas e tratamentos de controle de qualidade deve ser considerada. Sua diversidade e alterações em sua composição, justificada por inúmeros fatores, reforçam ainda mais essa necessidade. Adicionalmente, estudos de monitoramento sazonal da composição química podem auxiliar na busca por características que se diferenciam em determinas épocas do ano.

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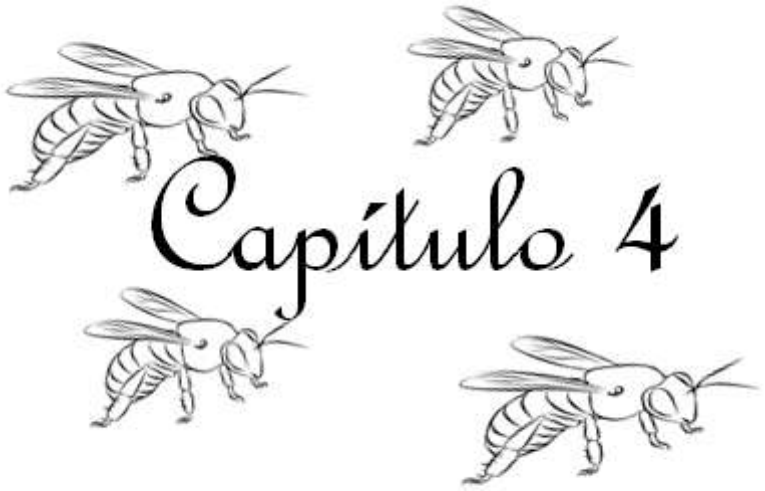

Molecular network-guided chemical profile and mass spectrometry, volatile compounds and antimicrobial activity of *Scaptotrigona depilis* propolis

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Artigo 4

Molecular network-guided chemical profile and mass spectrometry, volatile compounds and antimicrobial activity of *Scaptotrigona depilis* propolis

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Abstract

Rationale: Propolis has a great diversity in its composition due to numerous factors, therefore each study is an important contribution to the knowledge of its composition and biological action. The objective of this study was to determine the chemical profile and biological activity of propolis produced by *Scaptotrigona depilis*.
Methods: Extracts with 70% ethanol (EPE70) and with cereal alcohol (CAPE) were elaborated, and then characterized using UHPLC-ESI(+)-MS/MS. Volatile compounds were extracted and then characterized using GC-MS. In addition, antimicrobial activities were verified against resistant strains.
Results: The volatile compounds of propolis consist predominantly of sesquiterpenes. By means of the exploratory metabolomic approach, compounds of different classes were putatively identified in the ethanolic extracts, of which the most representative were terpenes, and some of the sesquiterpenes identified among the volatiles were also detected. The extracts were shown to be active against *E. coli* and *S. aureus* bacteria with a MIC of 0.5 mg mL^{-1} and 1.0 mg mL^{-1}, respectively.
Conclusions: The molecular network approach proved to be determining the chemical profile of *S. depilis* propolis rapidly and accurately, and led to the identification of lipophilic compounds. The identification of compounds by GC-MS and UHPLC-ESI(+)-MS/MS is complementary and useful for the characterization of propolis.

Keywords: *Stingless bee, metabolomics, molecular network, terpenes*.

1. INTRODUCTION

Stingless bee keeping has aroused interest for being a sustainable activity that allows us to generate products with a high market value from local biodiversity and in areas of environmental conservation[1,2]. Because of its peculiar characteristics, such as its diversity of flavors and bioactive properties, honey is one of the most relevant products today[3]. However, other by-products, such as pollen and propolis, also have great potential to be exploited and expand the income possibilities of stingless bee keepers [4-6].

The species of stingless bees of the genus *Scaptotrigona* stand out in meliponiculture throughout the Americas due to the resistance of the species in anthropized environments, the large population of the colonies, ease of multiplication, efficiency in agricultural pollination and good productivity of honey, pollen and propolis [7]. As a result of the great productive potential of the genus *Scaptotrigona*, its propolis has been investigated, to which different biological activities have been attributed [8-14]. In Brazil, *Scaptotrigona depilis*, popularly known as mandaguari, can be highlighted. It has a wide geographical distribution, well-known basic biology, and has been widely used for meliponiculture [15,16]. It is part of a complex of species that needs taxonomic revision and, therefore, is often referenced in the scientific literature as *S. postica* or *S.* aff *depilis* [16].

Because propolis has great variability in its composition, it is an inexhaustible source of research in relation to its chemical characterization. Studies aimed at the chemical characterization of propolis from stingless bees have identified fixed and volatile compounds. Mono- and sesquiterpenes, alkanes, aromatic compounds and fatty acids have already been identified in the volatile fraction [17-19]. In the characterization of extracts, prenylated benzophenones, lipophilic compounds (sesquiterpenes, diterpenes and triterpenes), as well as hydrocarbons, phenolic acids, flavonoids and esters have been noted [17-22].

The complexity of propolis is also related to the structural changes of molecules as a result of salivary enzymes, which are added in the production of this product [23]. In this way, the characterization of propolis in various places around the world is increasing. These studies support the search for specific characteristics in the chemical profile and biological activity of propolis in a certain place and time of year, thus aiding management and collection.

Therefore, the objective of this study was to determine the chemical composition of *Scaptotrigona depilis* propolis, through the identification of its volatile and fixed compounds, in addition to evaluating its antimicrobial potential.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

The collection of propolis occurred in three colonies that are kept in the meliponary at Embrapa Meio Ambiente, in Jaguariúna, SP, Brazil. The botanical origin of the propolis is diverse since the local flora at the site of collection is a semi-deciduous forest fragment that is predominant in the transition between the Cerrado and the Atlantic Rainforest biomes in southeastern Brazil. Samples were taken from propolis found under the lid of the hives. Each colony produced 423 g, 374 g and 520 g. The samples were then cooled and subsequently ground in a pestle and mortar.

2.2 Extraction of volatiles

For the extraction, 15.04 g of ground propolis were subjected to hydrodistillation for 3 h in a Clevenger apparatus. The hydrolate was collected and subjected to liquid-liquid extraction with ethyl acetate (3 x 5 mL). The organic phase was dried with anhydrous sodium sulfate and the solvent was concentrated for further analysis.

2.3 Gas chromatography-mass spectrometry analysis (GC-MS)

A gas chromatograph (Shimadzu model GC-2010) coupled to a mass spectrometer (Shimadzu model QP2010 Plus) was used for the analysis of volatile compounds. Separation was performed using a fused silica capillary column (RTX-5ms, 30 m × 0.25 mm × 0.25 μm). The injector temperature was 220 °C, the interface temperature was 280 °C, and the column temperature was programmed to increase from 60 °C to 280 °C at 3 °C min^{-1}. Helium was used as the carrier gas at a constant flow rate of 1.02 mL min^{-1}. The mass spectra were acquired in the *m/z* 40-600 range using electron ionization, with an ionization power of 70 eV, and the ion source at 260 °C. The composition of the essential oils was determined by comparing the values of their retention indices with those obtained for the homologous series of n-alkanes (C7-C30) under the same conditions, according to the method of Van den Dool and Kratz[24]. Subsequently, the experimental mass spectra were verified by comparison with those in the Wiley 8 and FFNSC 1.2 digital libraries and with data from existing literature [25].

2.4 Obtention of extracts

Portions of 2.13 g of ground propolis were used to prepare extracts using different solvents and under different conditions. Ethanolic extracts were elaborated in triplicate. The propolis portion was suspended in 100 mL of cereal alcohol 96% (CAPE), for 7 days, with periodic agitation. The same procedure was performed with 70% ethyl alcohol (EPE70). After this period, the solid material was separated from the solution by filtration, the solvent was removed at reduced pressure, and the obtained extracts (EPE70 and CAPE) were placed in a desiccator.

2.5 Monitoring of phenolic content during the extraction period

Portions of 0.25 g of ground propolis were used to prepare extracts for monitoring the phenolic content during the extraction period. An aliquot of the supernatant from the propolis suspension was taken daily for quantification of the phenolics using the method of Folin-Ciocalteu[26]. In a volumetric flask of 5 mL, 50 µL of supernatant, 300 µL of Folin-Ciocalteu reagent and 2.5 mL of sodium carbonate 5% were added. The solutions were placed under a light for a period of 2 hours. At the end of the reaction, the solutions were analyzed in a UV-VIS spectrophotometer (UVMini1240-Shimadzu) equipped with a 10 mm light path cell at 783 nm. To determine the concentration of the phenolics, a calibration curve with gallic acid was constructed within a concentration range of 2.0 x 10^{-3} to 1.2 x 10^{-2} mg mL^{-1}.

2.6 Ultra-high performance liquid chromatography–electrospray ionization-tandem mass spectrometry analysis (UHPLC-ESI(+)-MS/MS).

The analysis of the extracts was performed using ultra-high-performance liquid chromatography (Shimadzu, Nexera X2, Japan) coupled to a quadrupole time-of-flight high-resolution mass spectrometer (Impact II, Bruker Daltonics Corporation, Germany) equipped with an electrospray ionization source. Chromatographic separations were performed using a UPLC CHS C18 column (Acquity, Waters, USA, 1.7 µm, 2.1 × 100 mm) at a flow rate of 0.200 mL min^{-1}. The gradient mixture of solvents A (H_2O with 0.1% formic acid, v:v) and B (acetonitrile with 0.1% formic acid, v:v) was as follow: 2% B 0–1 min, 30% B 1–3 min, 80% B 3–20 min, 98% B 20–32 min and maintained at 2% B 32–38 min at 40 °C. The instrument was calibrated using a solution of sodium formate (10 mmol L^{-1} isopropanol:water; 1:1; v:v) containing 50 µL formic acid. The ionization source operated in the positive ionization mode and adjusted to 3500 V, with a potential plate end of −500 V. The dry gas parameters were set to 8 L min^{-1} at 200 °C with a nebulization gas pressure of 4 bar. Data were acquired in a range of m/z 50 to 1200 and an acquisition rate of 7 Hz. The five most

intense ions were selected for automatic fragmentation (AutoMS/MS). Data collection and processing were carried out using the Hystar Application software version 3.2 and Otof Control (Bruker Daltonics Corporation, Germany).

2.7 Molecular networking

The data files of mass spectrometry of the samples were transferred to the GNPS virtual platform server to generate the chemical maps (ID = 93d5a894207f4a6c87f9a765d0c65d64), according to the platform documentation[27], according to the methodology of Silva et al.[28]. The molecular network was generated so that the mass tolerance of the precursor ions was 0.02 Da, since this value influences the grouping of almost identical fragmentation spectra (MS/MS). For each group of MS/MS spectra acquired, the mass variation of the fragment ions, which can be displaced from their expected *m/z* values, was considered for grouping (consensus spectrum creation), and was stipulated as ± 0.02 Da. Rows (relations between nodes) were formed only if the cosine score was above 0.6, with a minimum correspondence of four peaks in the fragmentation spectrum. Molecular networking spectra were then compared with spectra from the GNPS spectral libraries such Mass bank, NIST14, and HMDB[29-32]. The same data parameters were applied to the sample spectra. Molecular network data were visualized using Cytoscape® software[33], in which the fragmentation spectra of the ions that presented similarity with the spectra of the libraries were manually confronted with the fragmentation spectra of the proposed compounds, and their mass errors calculated. The data were also evaluated using a Venn diagram that was created using all nodes from the molecular networking, that correspond to different molecular features presented to each propolis extract.

2.7.1 Data processing and Metabolites Identification

The *SmartFormula*® algorithm (DataAnalysis version 4.2, Bruker Daltonics, Germany) was used to assign the molecular formula of the detected ions, and the identification of metabolites was performed using public databases KEGG, ChEBI, MetFrag, GNPS, and literature data obtained from indexed sites. Finally, all metabolites were putatively identified using mass accuracy measure, and manually fragmentation spectra inspection.

2.8 Antimicrobial activity

Gram-positive bacteria *Staphylococcus aureus* INCQS 0057 (ATCC 43300), Gram-negative bacteria *Pseudomonas aeruginosa* INCQS 00025 (ATCC 15442), *Escherichia coli* INCQS 00051

(ATCC 13863) and the yeast *Candida albicans* (ATCC 90028) were used for antimicrobial tests. The strains were provided by Coleção de Microrganismos de Referência em Vigilância Sanitária (CMRVS, FIOCRUZ-INCQS, Rio de Janeiro, RJ, Brazil). The bacteria were cultured in brain-heart infusion medium (BHI) at 36 ± 1 °C for 24 h and the yeast was cultured in Sabouraud at 36 ± 1 °C for 36 h. Cultures were adjusted to 0.5 equivalent of the McFarland standard turbidity scale (10^5 CFU mL^{-1}). The minimum inhibitory concentration (MIC) values of the extracts were determined using the method of microdilution in broth, using 96 well-plates, according to a method established by the Institute of Clinical and Laboratory Standards [34]. The negative control was DMSO 10% and the positive controls were ampicillin and fluconazole. The extract concentrations tested were 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 and 0.0078 mg mL^{-1}. Each concentration was tested in triplicate and the MIC values were considered when the three repetitions obtained the same result (inhibition or no-inhibition).

3. Results and Discussion

3.1 Composition of volatiles obtained via hydrodistillation

The hydrodistillation yields demonstrated that *S. depilis* collects resins with low concentrations of volatiles. **Figure 1** shows the chemical profile of the volatile compounds. In the analysis of the volatiles obtained via GC-MS, 91.28% of the total composition of the extracted volatiles were identified (**Table S1**).

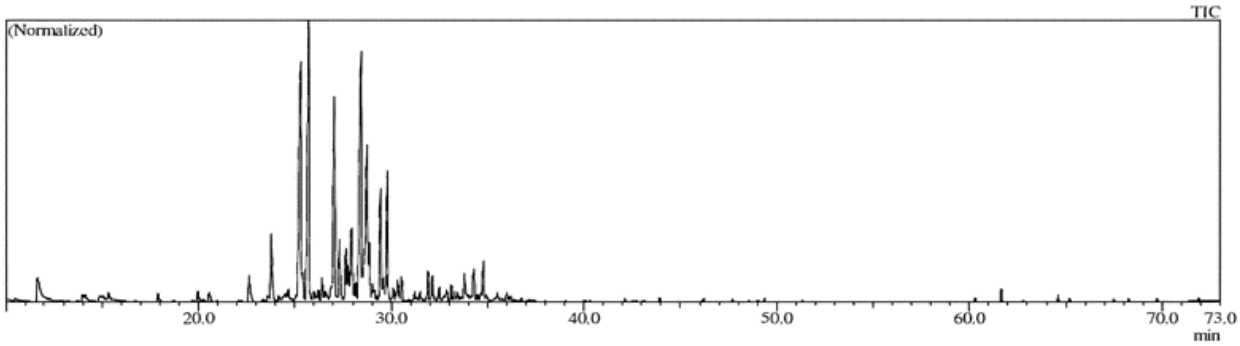

Figure 1- Chromatogram of total volatile ions (GC-MS) extracted from propolis using hydrodistillation.

The compounds with the highest levels were *cis*-β-farnesene (15.90%), *α*-guaiene (14.85%), α-selinene (14.41%), linalool isovalerate (8.88%) and α-bulnesene (7.65%), as shown in

Table S1. No data on the composition of propolis volatiles produced by *S. depilis* were found in the literature. Given the diversity of factors that influence the composition of this product, our study is an important contribution to the knowledge of the chemical composition of propolis of this species. In comparison with data for propolis produced by stingless bees, in the studies by Pino et al.[19], among the volatiles of the propolis of *Melipona beecheii*, cyclosativene, alloaromadendrene, germacrene A, *α*-cadinene, and *α*-cadinol were also noted. In studies conducted with the propolis of *Frieseomelitta longipes* and *Apis mellifera,* Souza et al.[17] identified *α*-guaiene and *α*-cadinene only in volatiles of propolis from *F. longipes*, while δ-gurjunene, cis-β-guaiene, and *trans*-cadine-1,4-diene were detected only in the propolis of *A. mellifera*. The presence of volatile compounds in propolis is common, and some studies point out that these constituents are olfactory clues to guide the collection of resin by bees [35-37].

3.2 Phenolic content

The amount of time taken for extraction is very important so that the extract obtained has the best characteristics as to its composition and biological activity. Therefore, the phenolic content was monitored to determine the optimal extraction period, and an aliquot was taken each day. The results were expressed in mg of gallic acid/g of propolis (**Table 1**). The cereal alcohol propolis extract (CAPE) provided the highest levels of phenolics, on the first day of extraction there was already a noticeable difference between the quantities displayed by the EPE70 and, at the end of the extraction, the content is more than double the value presented by the EPE70 extract.

Table 1- Phenolic content measured during extraction period.

Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
	mg of gallic acid/g of propolis													
EPE70	0.26	0.33	0.48	0.48	0.81	1.12	1.14	1.41	1.71	1.9	1.97	2.16	2.16	2.16
CAPE	0.70	1.29	1.65	2.38	2.6	3.06	3.80	3.90	3.95	4.05	4.32	4.83	4.98	5.00

Wozniak et al. [38] evaluated the relationship between the extraction solvent and the biological activity of Polish propolis and found that propolis extracts made with 96% ethanol present a more expressive phenolic concentration than the 70% extract. Despite this, both extracts exhibited good antioxidant, cytotoxic and antifungal activities.

3.4 Chemical profile of the extracts

The base peak chromatograms (BPC) of the *S. depilis* propolis extracts obtained from the analysis using UHPLC-ESI(+)-MS/MS can be seen in **Figure 2**. Differences in chromatographic profiles can be observed.

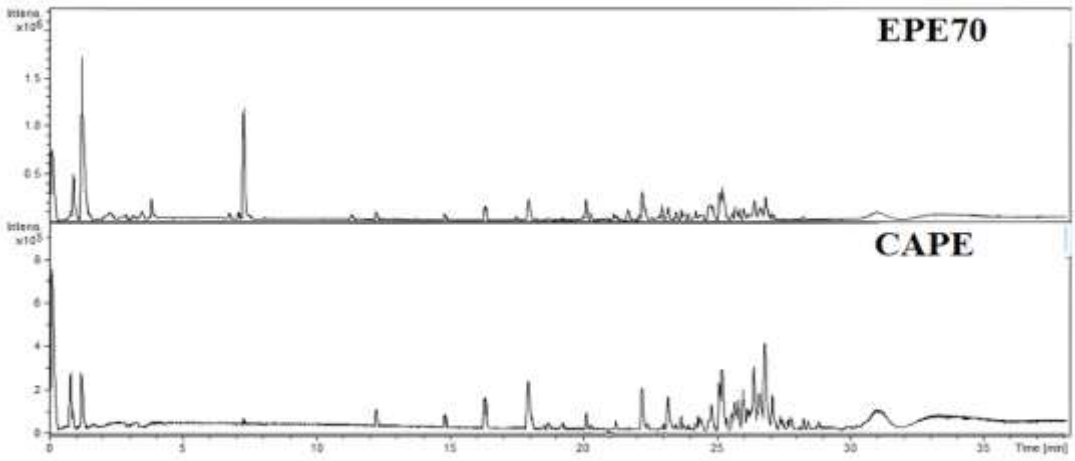

Figure 2- Base peak chromatograms (BPC) of the propolis extracts (EPE70 - ethanolic propolis extract 70% and CAPE - cereal alcohol extract 96%).

In the CAPE extract the incidence of peaks between 20 and 30 minutes is more representative, while EPE70 presents an eluted peak between 7.32 minutes that has greater relative abundance, and whose mass spectrum presents a molecular ion at *m/z* 219.114. This ion was provisionally identified as abrine, an amino acid, with an error of 3.19 ppm (**Table 2S**, **Figure 16S**).

3.5 Molecular network (MN)

After the treatment of the data on the GNPS platform, it was possible to evaluate the data by means of ensemble analysis. Thus, via Venn diagram (**Figure 3**), it is possible to determine the differences in the chemical profiles of propolis extracts more easily.

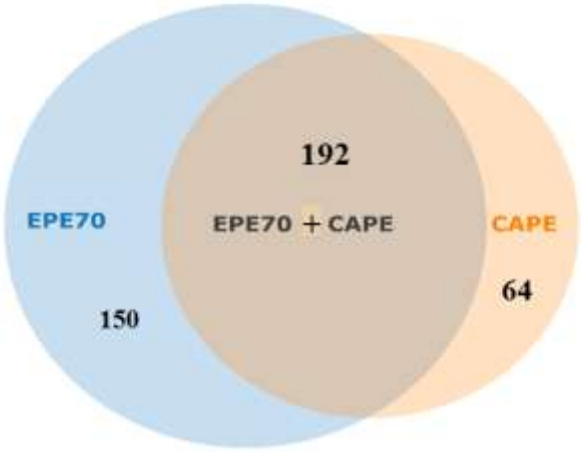

Figure 3- Venn diagram of the molecular features present in propolis extracts.

By analyzing the Venn diagram of propolis extracts, it was possible to obtain 406 molecular features, in which 192 of them are present in both extracts. The EPE70 has the highest number of detected chemical entities, 342, which is equivalent to 84.24% of the total detected entities. In addition, it has the highest number of unique chemical entities. From these data, 70% ethanol can be considered to be a solvent that is capable of extracting greater diversity of compounds compared to 96% cereal alcohol.

According to Silva[39], the most used solvent for the preparation of propolis extracts is ethyl alcohol, mainly food-grade alcohol, such as cereal alcohol. On the other hand, the Ministry of Agriculture, by means of Normative Instruction nº 11, of October 20, 2000 of the SDA/DIPOA, annex VII[40] establishes the use of ethyl alcohol 70% for propolis extract. Given the results obtained, it is observed that, in order to obtain an extract with a diverse composition, ethanol 70% is the most appropriate.

Regarding the molecular network (MN) of the EPE70 and CAPE extracts, a total of 417 nodes were obtained via the molecular network (**Figure 4**), which was grouped into 41 clusters, starting from those with 2 nodes. Through the Cytoscape® software, it was possible to adjust the attributes of the generated MN, such as the color and shape of the nodes. Hexagonal nodes represent the spectra that showed compatibility with the library.

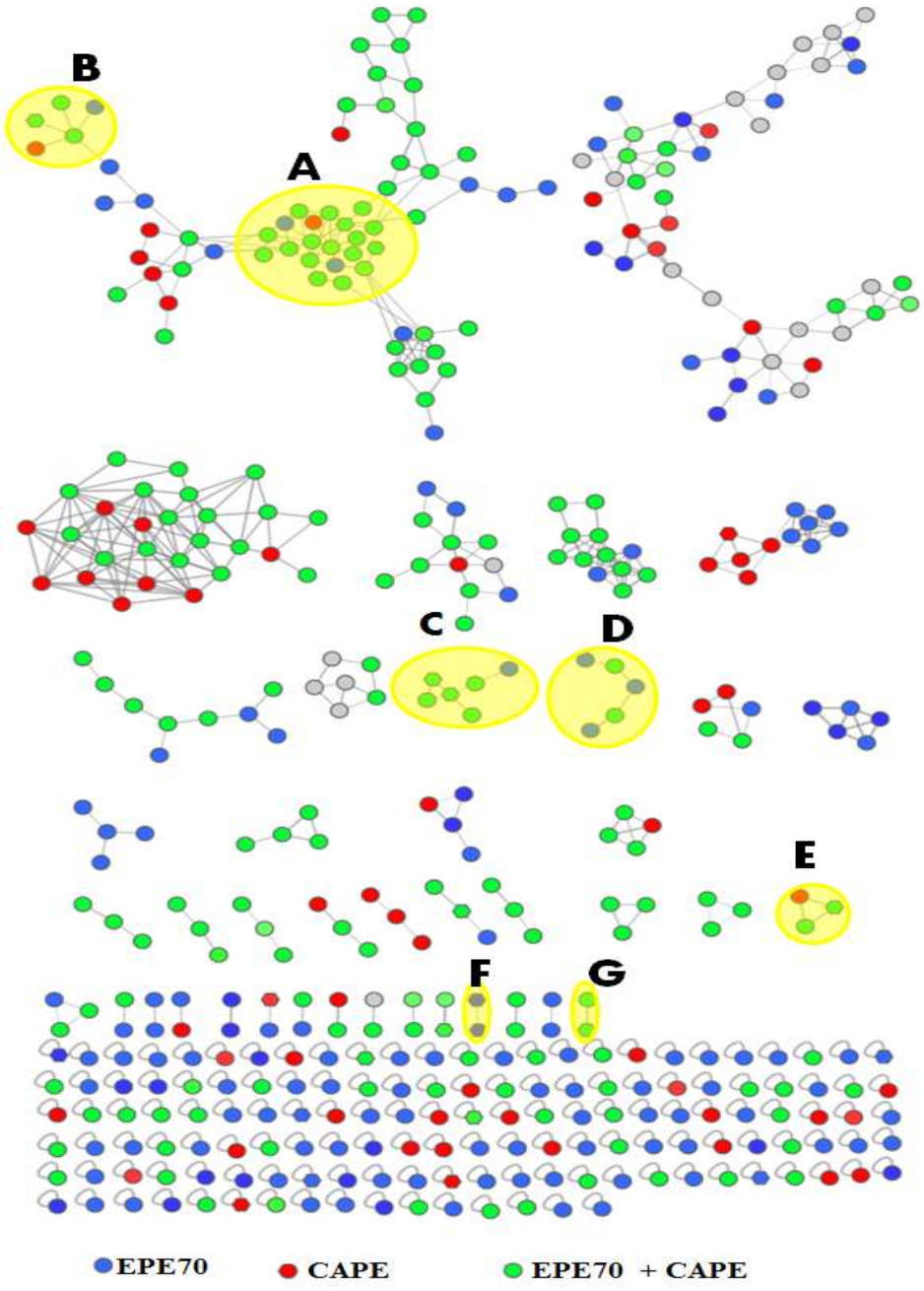

Figure 4- Molecular network map generated by GNPS, associated with MS/MS spectra obtained through the analysis of propolis extracts of *S. depilis*, in the positive mode of ionization. The hexagon-shaped nodes represent the MS/MS spectra that had hits with the spectra of the GNPS libraries. Nodes with putatively identified molecules are represented by letters: A) triterpenes, B) fatty acids cluster I, C) diterpenes, D) sesquiterpenes, E) fatty acids cluster II, F) amino acids and G) sphingolipids.

The putative annotation of metabolites was performed based on their fragmentation patterns compared to data from the literature and research in the GNPS spectral library, in addition to *in silico* assignments. A total of 41 compounds belonging to different classes were tentatively identified, including amino acids, terpenes, sphingolipids, fatty acids, phenolic acids and flavonoids (**Table S2**). Based on the attributions, it was observed that terpenes are the most representative compounds and are present in clusters with a significant number of nodes; many of them have already been cited in the composition of propolis of different origins. **Figure 5** represents the cluster of diterpenes present in the MN.

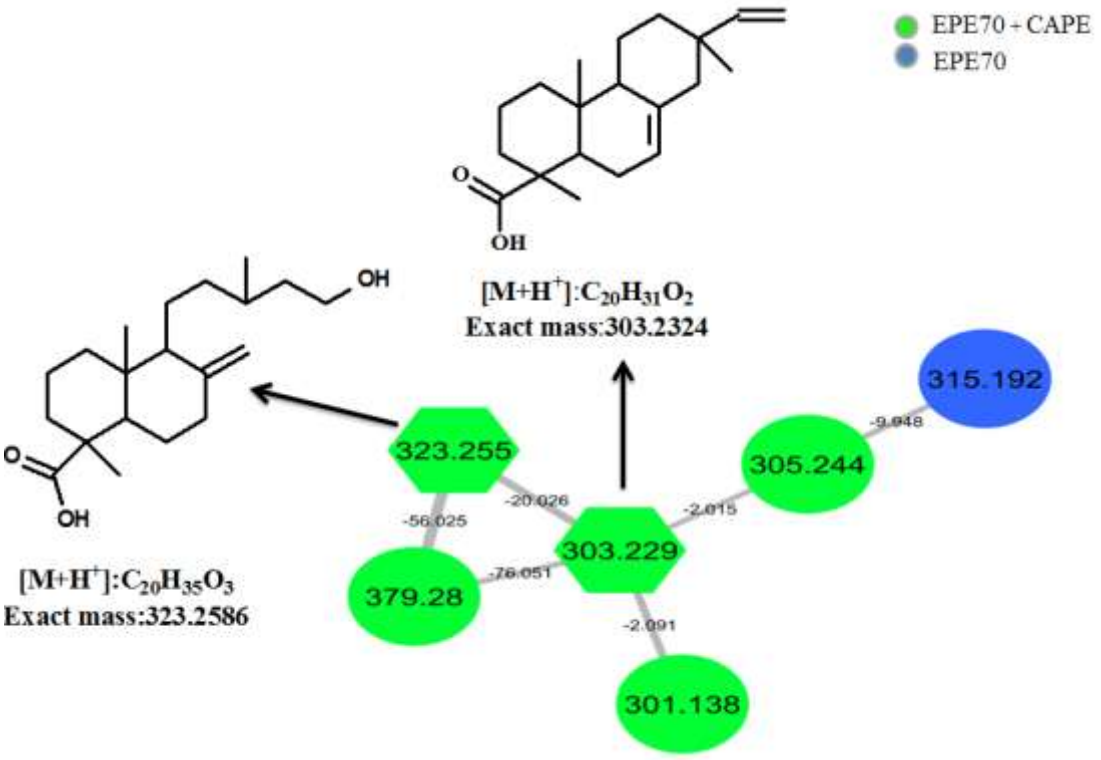

Figure 5- Representation of the cluster of diterpenes obtained from the molecular network generated by the GNPS site for the ethanolic extracts from the propolis of *S. depilis*. Nodes in blue correspond to the chemical entities present only in EPE70, nodes in green are entities present in EPE70 and CAPE, and hexagonal nodes represent the spectra of consensus with the GNPS site. The annotation in m/z 303.229 correspond to isopimaric acid and m/z 323.255 annotation correspond to (1R,4aS,5R,8aS)-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylidene-3,4,5,7,8,8a-hexahydro-2H-naphthalene-1-carboxylic acid.

The hexagonal nodes represent the compounds that presented similarity with the GNPS library, and are the *m/z* 303.229 and *m/z* 323.255 ions, separated by 20.028 Da. The annotation made by the library suggests that they are isopimaric acid (*m/z* 303.229, $C_{20}H_{31}O_2$) and (1*R*,4*aS*,5*R*,8*aS*)-5-(5-hydroxy-3-methylpentyl)-1,4*a*-dimethyl-6-methylidene-3,4,5,7,8,8*a*-hexahydro-2*H*-naphthalene-1-carboxylic acid (*m/z* 323.2550, $C_{20}H_{35}O_3$). The mass spectrum of the *m/z* 303.229 ion is characterized by the presence of the base peak at *m/z* 257.226, which is compatible with the loss of the M-COOH group. Rahman et al.[41], in their proposal for EI-MS fragmentation for isopimara-7,15-dien-19-oic acid, a stereoisomer of of isopimaric acid, suggest that the ion at *m/z* 257 comes from decarboxylation. The presence of a double bond in the B-ring between C-7 and C-8 would facilitate a reaction of the Retro Diels Alder (RDA) type, cleaving the B ring. Demarque et al.[42] also discussed more common fragmentation mechanisms for ions generated by ESI-MS including the RDA. Based on the literature, **Figure 6** illustrates a proposal for the formation of some isopimaric acid fragments observed in the mass spectrum (**Figure S28**).

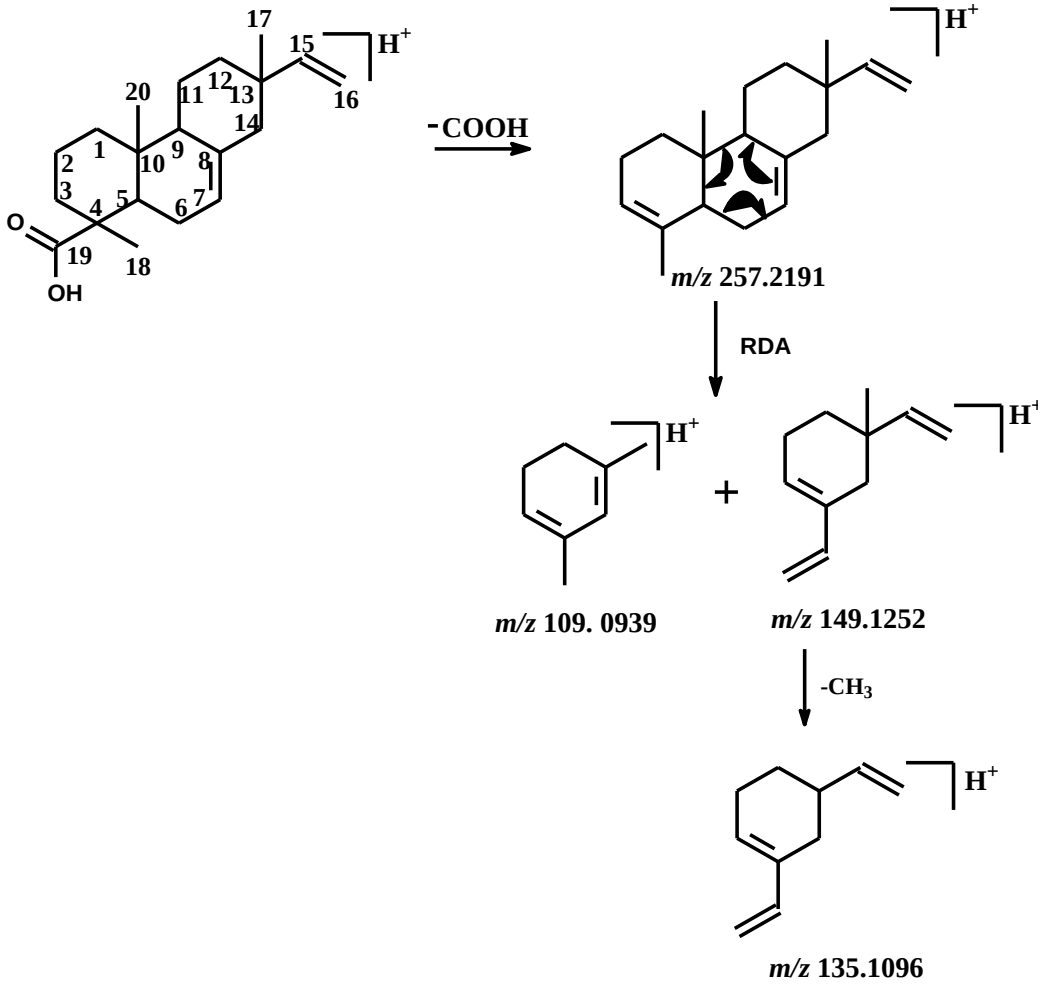

Figure 6- Proposed fragment formation for isopimaric acid.

The presence of some of these diterpenes in propolis is reported in the literature. Kartal et al.[43] characterized the honey produced by *A. mellifera* in two towns in Turkey using GC-MS, and identified isopimaric acid in both samples, the presence of these constituents is associated with the exsudate of *Pinus brutia* L. Popova et al.[44] studied the propolis from Turkey and also identify isopimaric acid. These same compounds have also been identified in propolis from Greece, whose plant origin has been attributed to a species of *Pinus* sp[45].

Aminimoghadamforouj and Nematollahi[46] gathered data from studies that performed fractionation of propolis extracts resulting in the isolation of, among other compounds, diterpenes, with potential for the discovery of new drugs since they present biological action.

Another subclass of compounds that presented a significant number of annotations were triterpenes. The cluster that contains them has the largest number of nodes. In **Figure 7**, a portion of the triterpene cluster is represented.

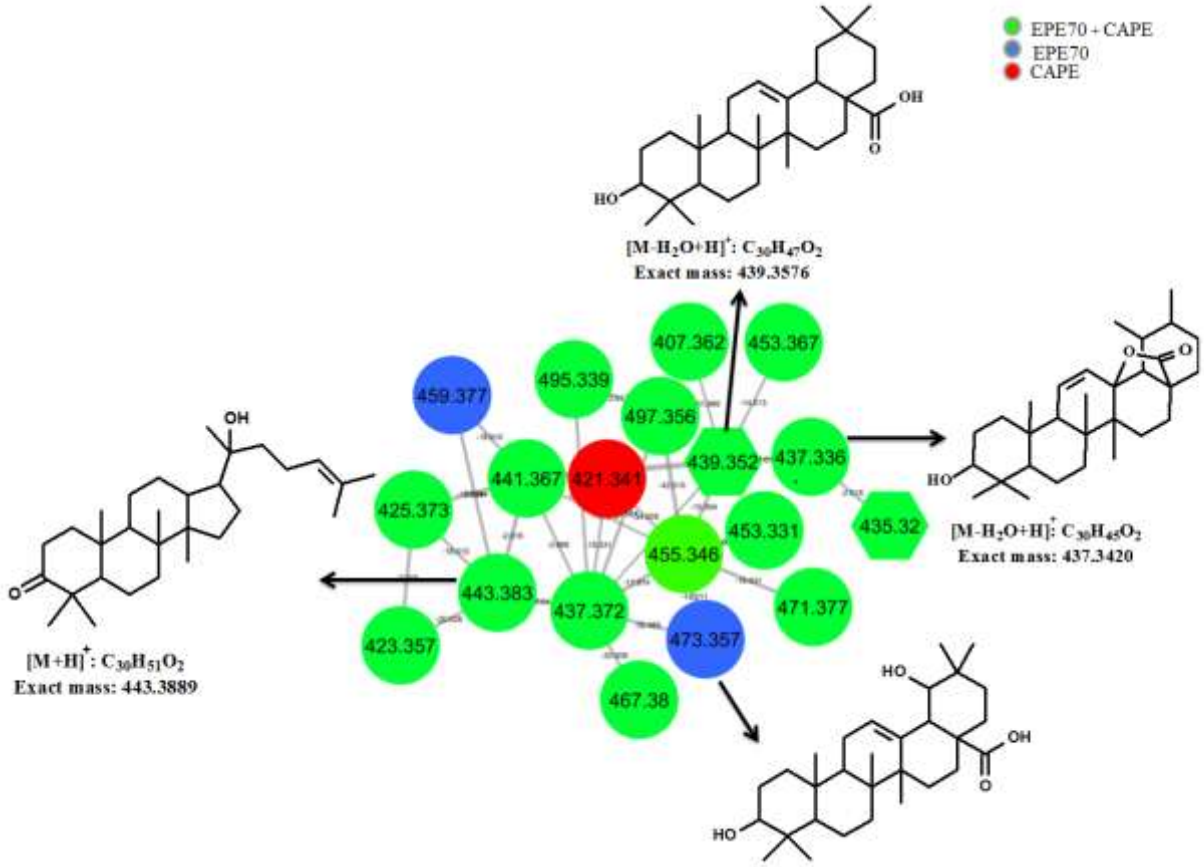

Figure 7- Representation of the triterpene cluster obtained from the molecular network generated by the GNPS site from the MS/MS data of the propolis extracts of *S. depilis*. Nodes in blue correspond to the chemical entities present only in the extract EPE70, nodes in green are entities

present in the extracts EPE70 and CAPE, node in red correspond to the chemical entity present only in the extract CAPE and hexagonal nodes represent the spectra of consensus with the GNPS site.

With the exception of the *m/z* 473.357, 421.341 and 459.377 ions, the other compounds noted are present in both extracts. Demarque et al.[42] highlight the dehydration reaction that occurs in triterpenes in structures containing a hydroxyl group (-OH). According to the authors, water is eliminated with the consequent formation of a π bond. The presence of a π bond in the C ring suggests RDA. Based on this information, **Figure 8** presents a fragmentation proposal for the *m/z* 473.361 ion, allegedly identified as spinosic acid A (mass spectrum in **Figure S41**).

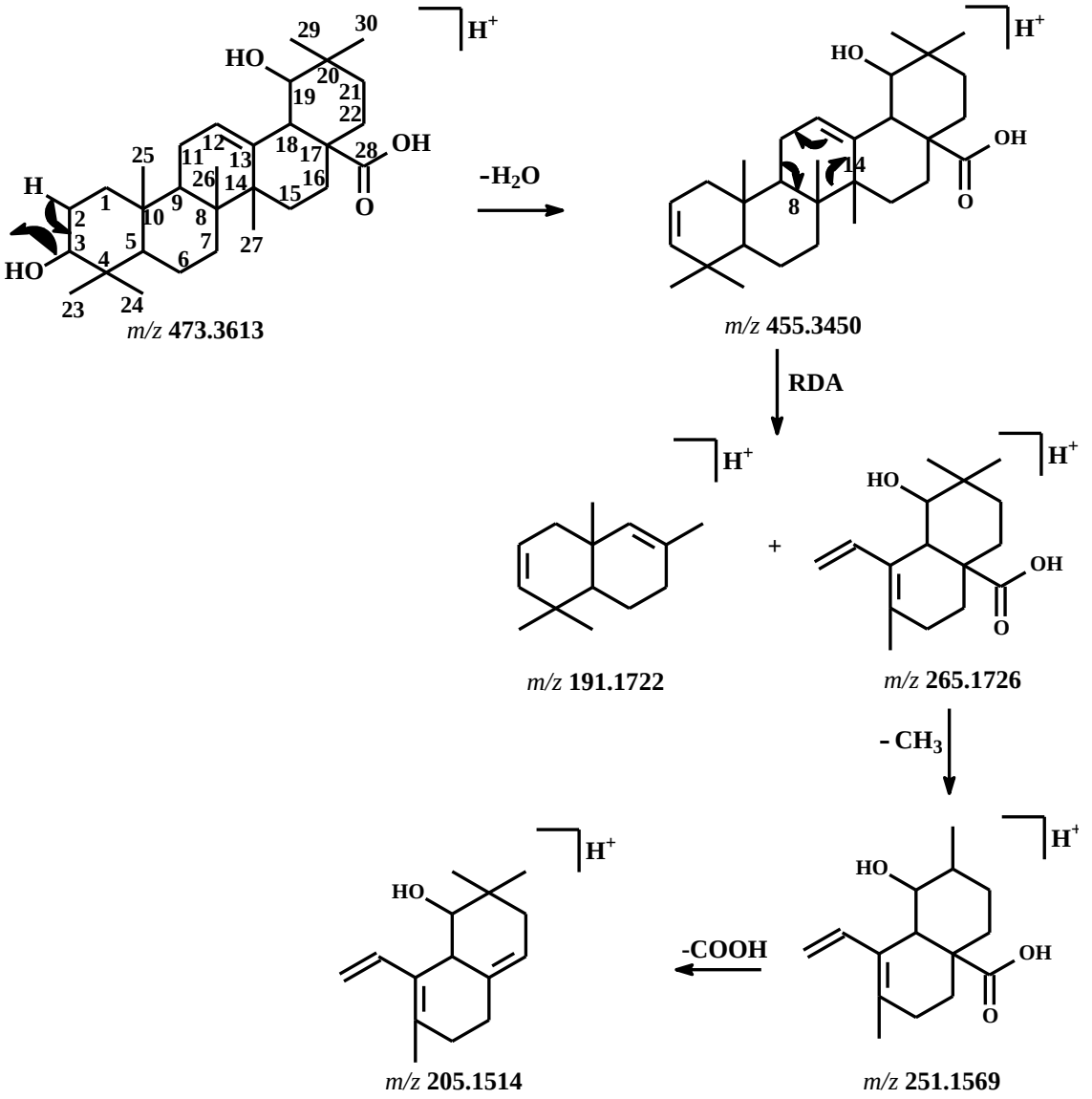

Figure 8. Proposed formation of some fragments of spinosic acid A.

The suggestions for the formation of the fragments for the structures are important for the substantiation of the notes. The identification attempts are also supported by the error (ppm) calculated, taking into account the difference in theoretical and experimental monoisotopic masses, in which all assigned compounds have errors below 5 ppm (**Table S2**).

The presence of triterpenes in propolis is cited in several works in the literature. Kardar et al.[47], in studies with propolis from Indonesia, cites that fractionation led to a mixture of compounds, of which nine were triterpenes. Pentacyclic triterpenes were obtained from the fractionation of the chloroform-methanolic extract of *Melipona beecheii* propolis, from Mexico[48]. The propolis produced by *Tetragonula sapiens* showed a predominance of cycloarthanes, and the investigation of the botanical origin showed similarities in the chromatographic profile of propolis extracts and with the ethanolic extract of *Mangifera indica*[49]. In propolis of Thailand, damaran-type triterpenes have been identified, among them, dipterocarpol[50], also putatively identified in the propolis of *S. depilis* (mass spectrum in **Figure S40**).

Eleven sesquiterpenes were also noted, of which six were present among the volatiles characterized using GC-MS (**Table S1**). The presence of these constituents that were detected by both techniques serve as the basis for supporting putative identifications.

Composed of a mixture of wax, resin and, depending on the species of bee, clay, propolis has a predominant composition of lipophilic constituents. In studies conducted with different materials of a hive of *Frieseomelitta silvestrii*, among them propolis, Netto et al.[20] identified sesquiterpenes, diterpenes and triterpenes in dichloromethane extract, and the chromatogram of total ions presents a clear separation of these different subclasses. However, Silva-Júnior et al.[51], in a non-targeted analysis with the data obtained by LC-MS, investigated the composition of different materials collected in colonies of *Scaptotrigona depilis*, including propolis, and cite the flavonoids as being the most representative compounds. Taking into account the normative quality standard of propolis, it is assumed that there are low concentrations of flavonoids; since the established minimum is 0.5%[52].

3.6 Antimicrobial activity

The ethanolic extracts (EPE70 and CAPE) were active against *E. coli* and *S. aureus*, and **Table 2** lists the minimum inhibitory concentrations.

Table 2- Minimum inhibitory concentrations of *S. depilis* propolis extracts against pathogenic bacteria.

	MIC (mg mL^{-1})			
Propolis Extract	*E. coli*	*S. aureus*	*P. aeruginosa*	*C.albicans*
EPE70	0.5	NI	NI	NI
CAPE	NI	1	NI	NI
Ampicillin	0.0078	0.0156	0.0625	NT
Fluconazole	NT	NT	NT	0.0313

Key: NI - no inhibition NT- not tested

For the strains *Pseudomonas aeruginosa* INCQS 00025 (ATCC 15442) and *Candida albicans* (ATCC 90028), the extracts were unable to inhibit growth. Although the results obtained for the samples are higher than the standard used, it should be considered that the strains used have resistance and that the test was performed with extracts with hundreds of compounds that may or may not have antimicrobial activity. The ATCC 13863 strain of *E. coli* used has resistance to phage T1 (a type of virus that infects only bacteria), and the ATCC 43300 strain of *S. aureus* is resistant to methicillin and oxacillin, so the MIC values of the extracts are considered significant.

It is known from the literature that propolis has antimicrobial activity and the study of products that may be useful in the discovery of new alternatives to antibiotics is important. Studies show that compounds identified in the extracts examined have antimicrobial potential. Isopimaric acid has already demonstrated inhibitory action of multidrug-resistant *S. aureus* [53], although it may cause liver dysfunction in fish. Triterpene damaran, dipterocarpol, isolated from *Tetrigone melanoleuca* propolis, showed activity against several strains, including *E. coli* and *S. aureus* [50].

4. CONCLUSIONS

The volatiles present in the propolis *of S. depilis* are predominantly sesquiterpenes, and some of them have also been identified using LC-MS. Ethanol in the commercial form of cereal alcohol was the most efficient solvent in the extraction of phenolic compounds, while 70% ethanol proved to be quite efficient in the extraction of several unique chemical entities. The profile of both

extracts presents a predominance of terpenes, whose occurrence is reported in the literature, though have not yet been identified for the propolis of *S. depilis*. The extracts have been shown to be active against two very important human pathogen multiresistant. The findings of this study provide new insights about the chemical composition of propolis from stingless bee *S. depilis*. We believe that it will be a great contribution to the meliponiculture production chain, a sustainable activity with the potential to generate income in preserved areas.

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SUPPORTING INFORMATION

Supporting Information**: Table S1** and **S2**, mass spectrum of the assigned compounds **Figure S1-S41.**

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Supplementary Material

Molecular network-guided chemical profile and mass spectrometry, volatile compounds and antimicrobial activity of *Scaptotrigona depilis* propolis

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Table S1- Constituents identified in volatiles extracted from propolis

Compounds	**RI_C**	**RI_d**	**Area (%)**
Cyclosativene	1366	1371	1.21
α-Duprezianene	1393	1388	2.84
α-Guaiene	**1431**	**1439**	**14.85**
cis-Thujopsene	1433	1431	0.90
***cis-β*-Farnesene**	**1441**	**1442**	**15.90**
Alloaromadendrene	1458	1458	0.74
Linalool isovalerate*	**1474**	**1468**	**8.88**
δ-Gujunene	1480	1477	2.36
α-Amorfene	1489	1484	1.85
cis-β-Guaiene	1496	1493	3.60
α*-Selinene	**1508**	**1498**	**14.41**
Gernacrene A*	1513	1509	2.16
***α*-Bulnesene**	**1516**	**1509**	**7.65**

α-Cadinene	1535	1538	4.33
trans-Cadina-1,4-diene	1544	1534	5.59
β-Calacorene*	1563	1564	0.76
β-Atlantol*	1598	1608	0.89
Himachalol	1663	1653	0.89
α-Cadinol*	1676	1654	1.47

Key: RI_C: Calculated retention index, RI_d: Retention index database, * Compounds also detected by LC-MS.

Table S2- Putative identification of metabolites in ethanolic extracts of propolis.

	Assigned metabolite	Molecular Formula	$M+H^+$ measured	$M+H^+$ theoretical	Mass accuracy (ppm)	Adduct	EPE70	CAPE
1	Pipecolic acid[a]	$C_6H_{11}NO_2$	130.0860	130.0863	-2.31	$M+H^+$	x	
2	L-Norleucine[a]	$C_6H_{13}NO_2$	132.1019	132.1019	0	$M+H^+$	x	
3	Trigonelline[a]	$C_7H_7NO_2$	138.0546	138.0549	-0.72	$M+H^+$	x	x
4	Limonene-1.2-epoxide[b]	$C_{10}H_{16}O$	153.1271	153.1274	-1.96	$M+H^+$	x	
5	Ricinine[a]	$C_8H_8N_2O_2$	165.0661	165.0664	-1.84	$M+H^+$	x	
6	L-Phenylalanine[a]	$C_9H_{11}NO_2$	166.0866	166.0863	1.81	$M+H^+$	x	
7	(R)-(-)-Mellein[a]	$C_{10}H_{10}O_3$	179.0694	179.0703	-5.03	$M+H^+$	x	
8	L-Tyrosine[a]	$C_9H_{11}NO_3$	182.0812	182.0812	0	$M+H^+$	x	
9	β-Calacorene[b]	$C_{15}H_{20}$	201.1639	201.1638	0.50	$M+H^+$	x	x
10	Leucyl-Alanine[a]	$C_9H_{18}N_2O_3$	203.1387	203.1390	-1.48	$M+H^+$	x	x
11	α-Curcumene[b]	$C_{15}H_{22}$	203. 1793	203.1794	-0.49	$M+H^+$	x	x
12	Germacreno A[b]	$C_{15}H_{24}$	205.1947	205.1950	-1.46	$M+H^+$	x	x
13	α-Selinene[b]	C15H24	205.1954	205.1950	1.94	$M+H^+$	x	x
14	L-Tryptophan[a]	$C_{11}H_{12}N_2O_2$	205.0976	205.0971	2.44	$M+H^+$	x	
15	α-Bisabol[a]	$C_{15}H_{26}O$	205.1955	205.1950	-1.46	$M-H_2O+H^+$	x	x
16	Abrine[a]	$C_{12}H_{14}N_2O_2$	219.1350	219.1128	3.19	$M+H^+$	x	x
17	Nootkatone[b]	$C_{15}H_{22}O$	219.1748	219.1743	2.28	$M+H^+$	x	x
18	α-Cyperol[b]	$C_{15}H_{24}O$	221.1897	221.1899	-0.9	$M+H^+$	x	x
19	Isocyperol[b]	$C_{15}H_{24}O$	221.1900	221.1899	0.45	$M+H^+$	x	
20	α-Atlantol[b]	$C_{15}H_{24}O$	221.1899	221.1899	0	$M+H^+$	x	x
21	α-Cadinol[b]	$C_{15}H_{24}O$	221.1903	221.1899	1.81	$M+H^+$	x	x
22	Linalool isovalerate[b]	$C_{15}H_{26}O_2$	239.2005	239.2006	-0.42	$M+H^+$	x	
23	*cis*-9-Hexadecenoic acid[a]	$C_{16}H_{30}O_2$	255.2317	255.2318	-0.39	$M+H^+$	x	x
24	Genistein[a]	$C_{15}H_{10}O_5$	271.0598	271.0601	-1.11	M+H	x	x

25	Dehydroabietadienal[a]	$C_{20}H_{28}O$	285.2210	285.2212	-0.7	M+H	x	x
26	3-Methyl-5-[(1S.8aS)-5.5.8a-trimethyl-2-methylenedecahydro-1-naphthalenyl]-2-pentenoic acid[a]	$C_{20}H_{30}O$	287.2375	287.2369	4.49	$M-H_2O+H^+$	x	x
27	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-9-hydroxy-1,4a-dimethyl-7-(1-methylethyl) [a]	$C_{20}H_{26}O_2$	299.2001	299.2005	-1.34	$M-H_2O+H^+$	x	x
28	Isopimaric acid[a]	$C_{20}H_{30}O_2$	303.2318	303.2318	0	$M+H^+$	x	x
29	*cis*-8,11,14-Eicosatrienoic acid[a]	$C_{20}H_{34}O_2$	307.2633	307.2631	0.65	$M+H^+$	x	x
30	Linoleic acid ethyl ester[a]	$C_{20}H_{34}O_2$	309.2790	309.2788	1.15	$M+H^+$	x	x
31	Oleic acid ethyl ester[a]	$C_{20}H_{38}O_2$	311.2950	311.2645	1.61	$M+H^+$	x	x
32	Dehydrophytosphingosine[b]	$C_{18}H_{37}NO_3$	316.2846	326.2846	0	$M+H^+$	x	x
33	Phytosphingosine[a]	$C_{18}H_{39}NO_3$	318.3009	318.3003	1.32	$M+H^+$	x	x
34	(E)-5-(1,2,4a,5-tetramethyl-7-oxo-3,4,8,8a-tetrahydro-2H-naphthalen-1-yl)-3-methylpent-2-enoic acid[a]	$C_{20}H_{30}O_3$	319.2265	319.2267	-0.63	$M+H^+$	x	x
35	Naphthalenecarboxylic acid, decahydro-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylene-, (1R,4aS,5R,8aS)- [a]	$C_{20}H_{34}O_3$	323.2581	323.2581	0	$M+H^+$	x	x
36	Hydroquinidine[a]	$C_{20}H_{26}N_2O_2$	327.2073	327.2067	0.16	$M+H^+$	x	
37	Mangiferin[a]	$C_{19}H_{18}O_{11}$	423.0922	423.0922	0	$M+H^+$	x	
38	3-Hydroxy-11-Ursen-28.13-Olide[a]	$C_{31}H_{48}O$	437.3420	437.3778	4.47	$M-H_2O+H^+$	x	x
39	Oleanolic acid [a]	$C_{30}H_{48}O_3$	439.3576	439.356	-4.79	$M-H_2O+H^+$	x	x
40	Dipterocarpol[a]	$C_{30}H_{50}O_2$	443.3876	443.3883	-1.57	$M+H^+$	x	
41	Spinosic acid A[b]	$C_{30}H_{48}O_4$	473.3613	473.3625	-2.53	$M+H^+$	x	

Key: [a] Identification through comparison of the mass spectrum with the GNPS library; [b] MetFrag.

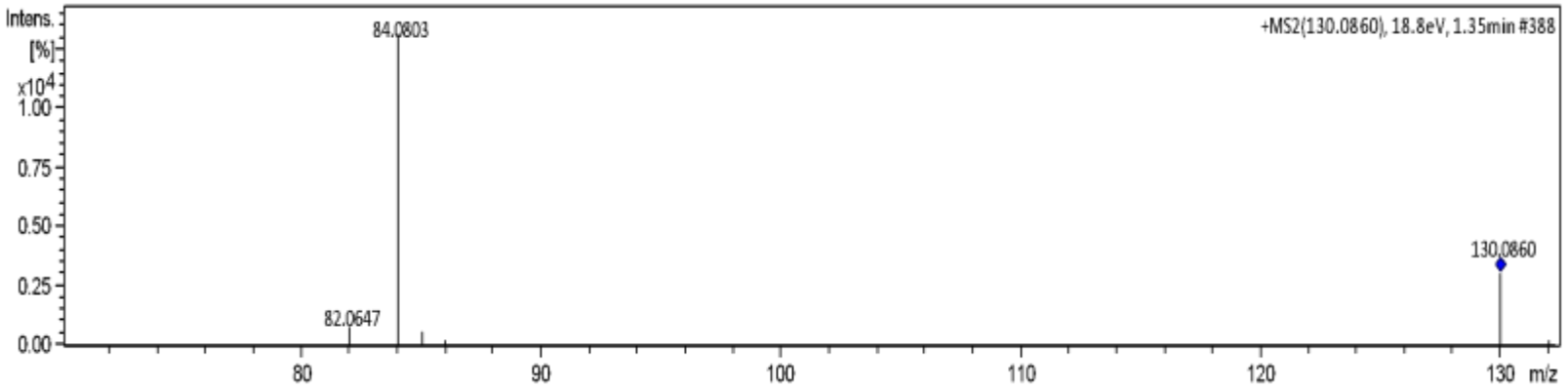

Figure S1- Mass spectra of the $M+H^+$ 130.0860 ion annotated as pipecolic acid present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

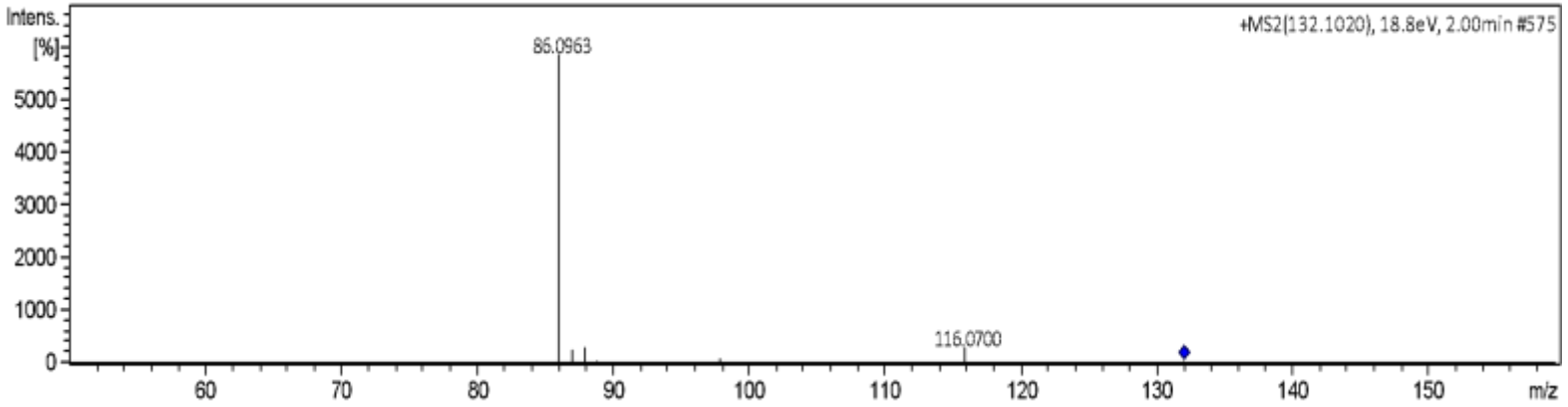

Figure S2- Mass spectra of the $M+H^+$ 132.1020 ion annotated as L-norleucine present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

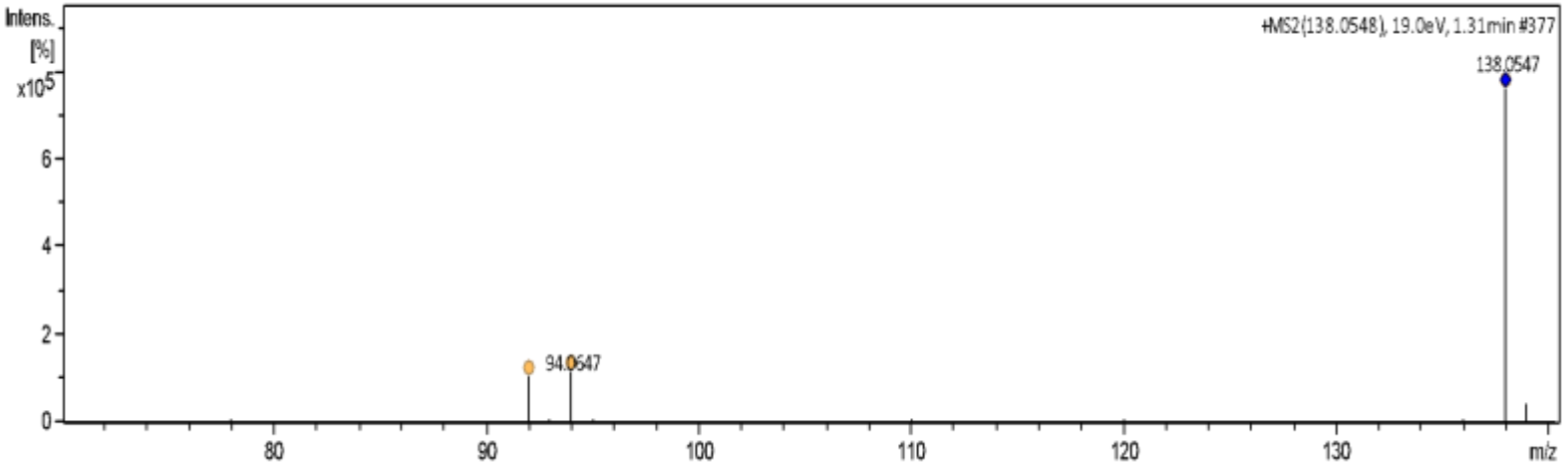

Figure S3- Mass spectra of the $M+H^+$ 138.0546 ion annotated as trigonelline present in the propolis extracts of *S. depilis* using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

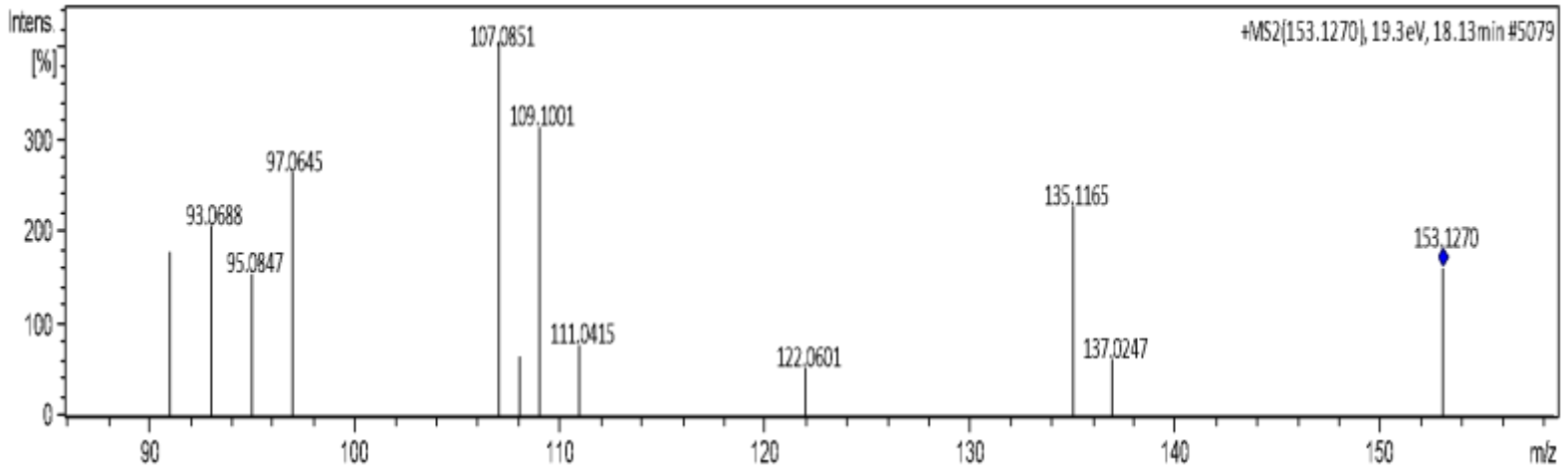

Figure S4- Mass spectra of the $M+H^+$ 153.1271 ion annotated as limonene-1,2-epoxide present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

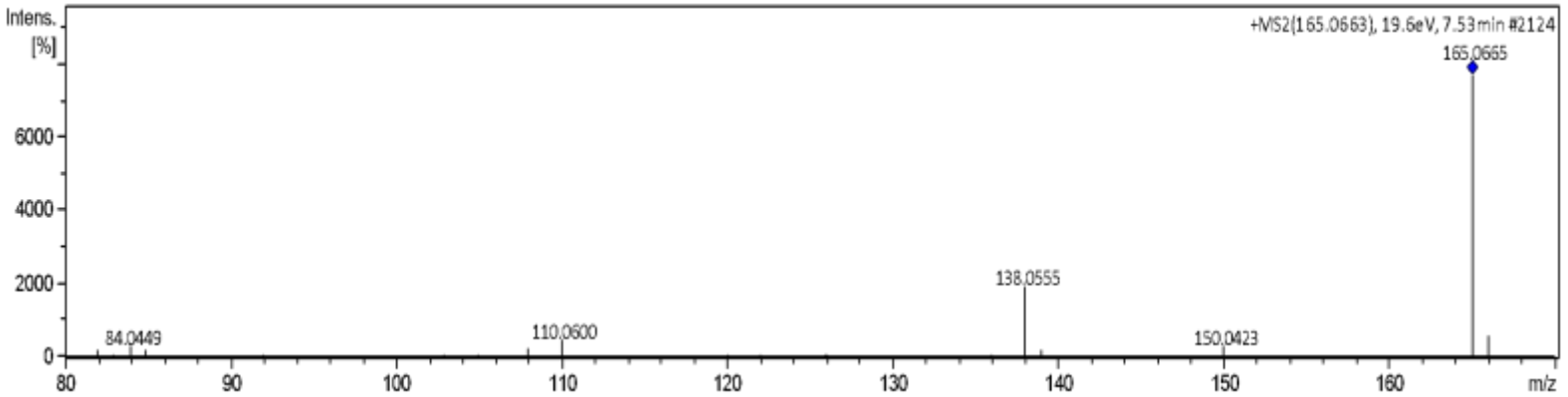

Figure S5- Mass spectra of the $M+H^+$ 165.0661 ion annotated as ricinine present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

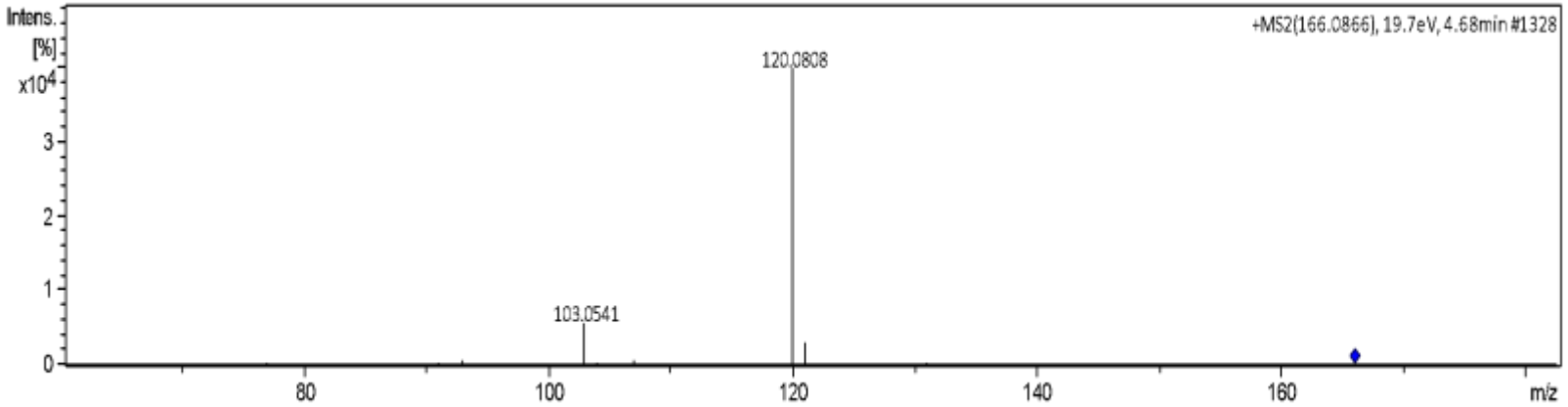

Figure S6- Mass spectra of the $M+H^+$ 166.0866 ion annotated as L-phenylalanine present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

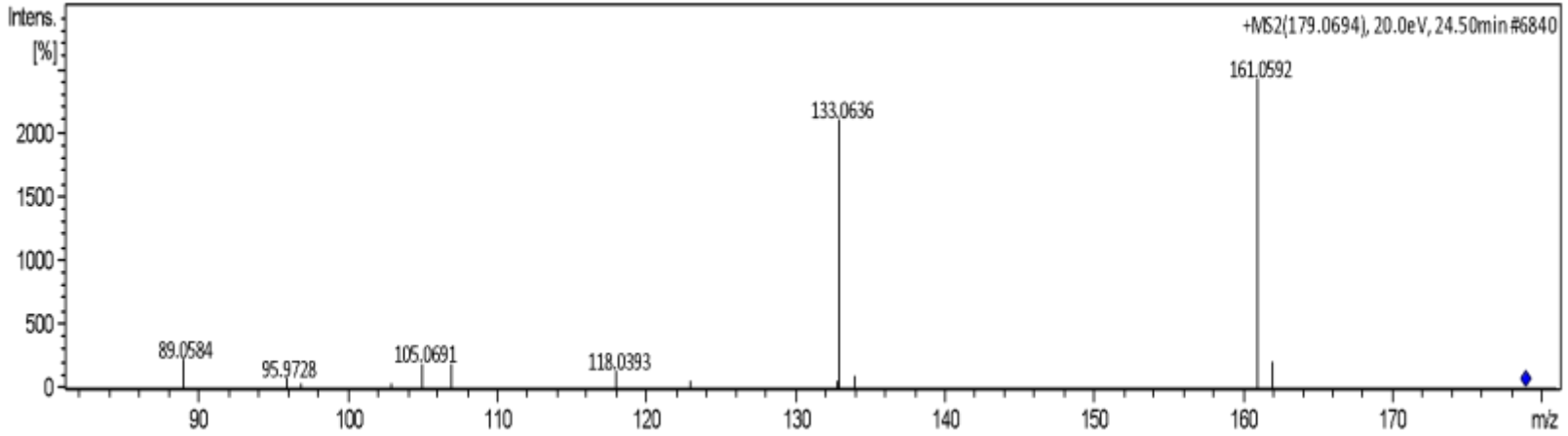

Figure S7- Mass spectra of the $M+H^+$ 179.0694 ion annotated as (R)-(-)-mellein present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

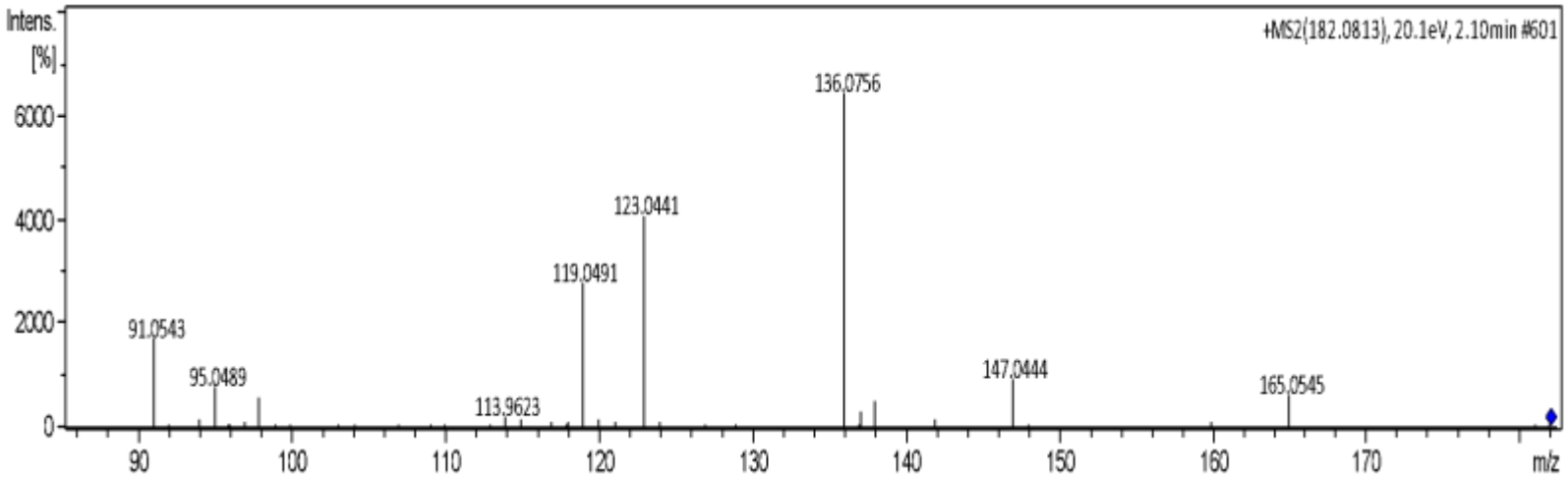

Figure S8- Mass spectra of the $M+H^+$ 182.0813 ion annotated as L-tyrosine present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

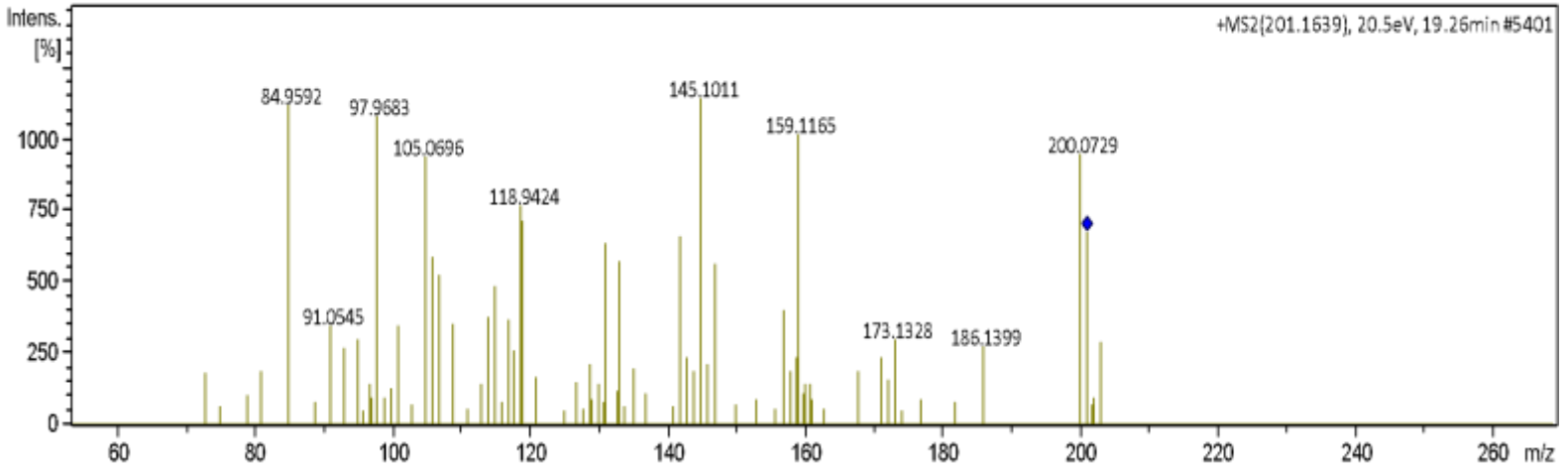

Figure S9- Mass spectra of the $M+H^+$ 201.1639 ion annotated as β-calacorene present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

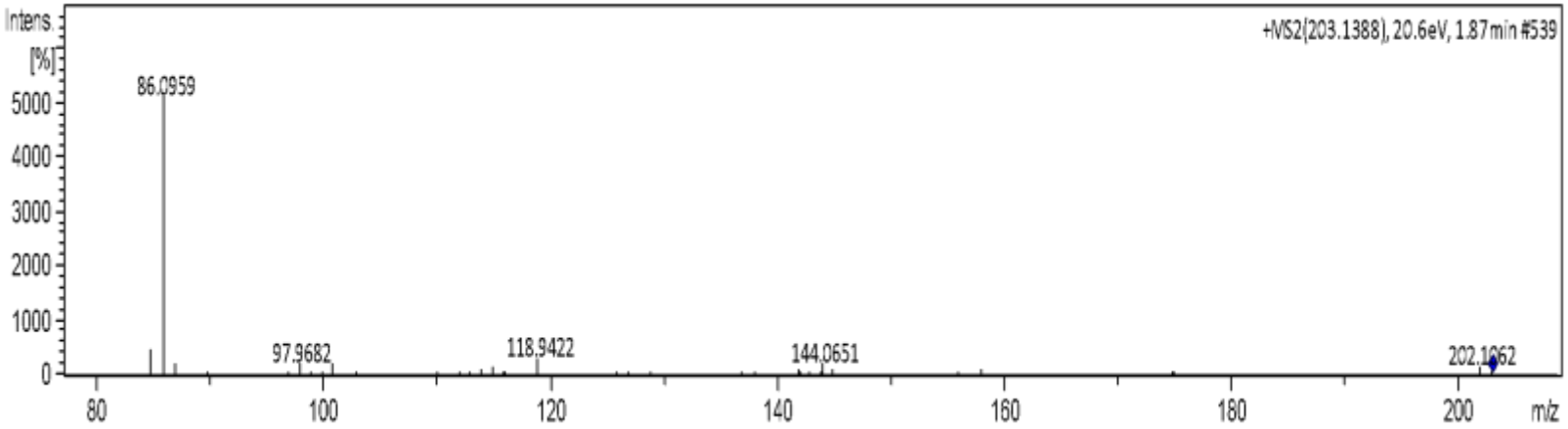

Figure S10- Mass spectra of the $M+H^+$ 203. 1388 ion annotated as leucyl-alanine present in *S. depilis* propolis extracts using data obtained from ultra-high efficiency liquid chromatography analysis coupled to a high resolution spectrometer.

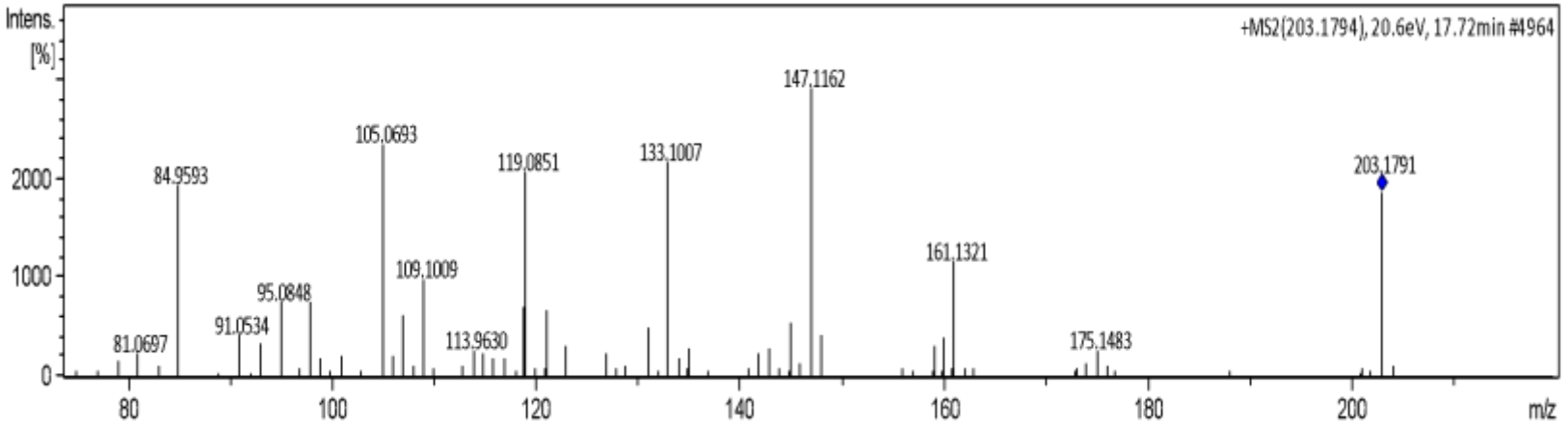

Figure S11- Mass spectra of the $M+H^+$ 203.1794 ion annotated as α-curcumene present in the *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

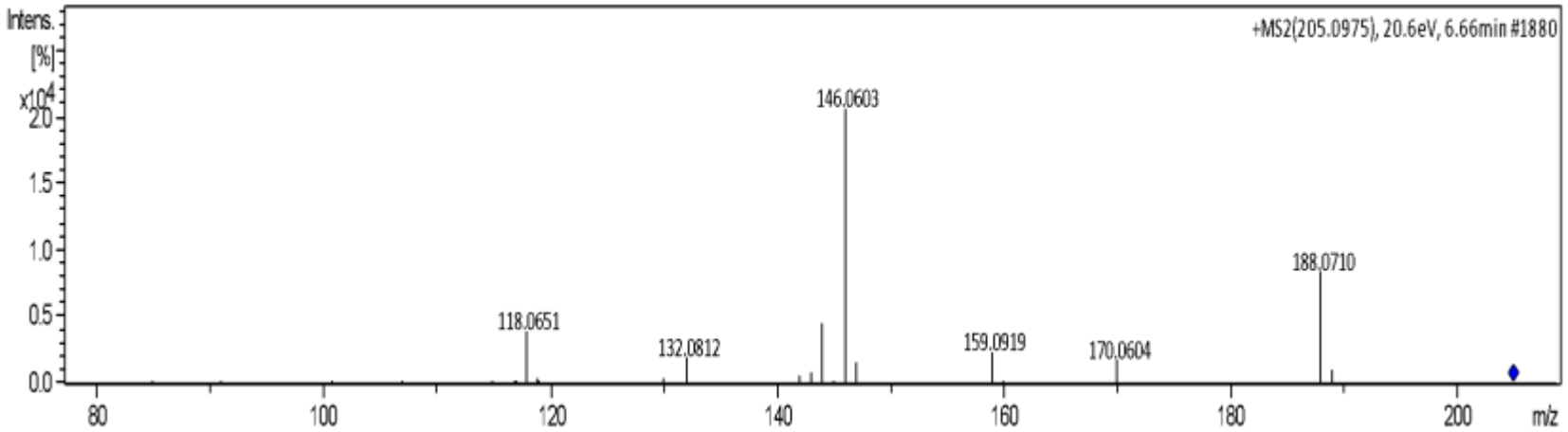

Figure S12- Mass spectra of the $M+H^+$ 205.0975 ion annotated as L-tryptophan present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

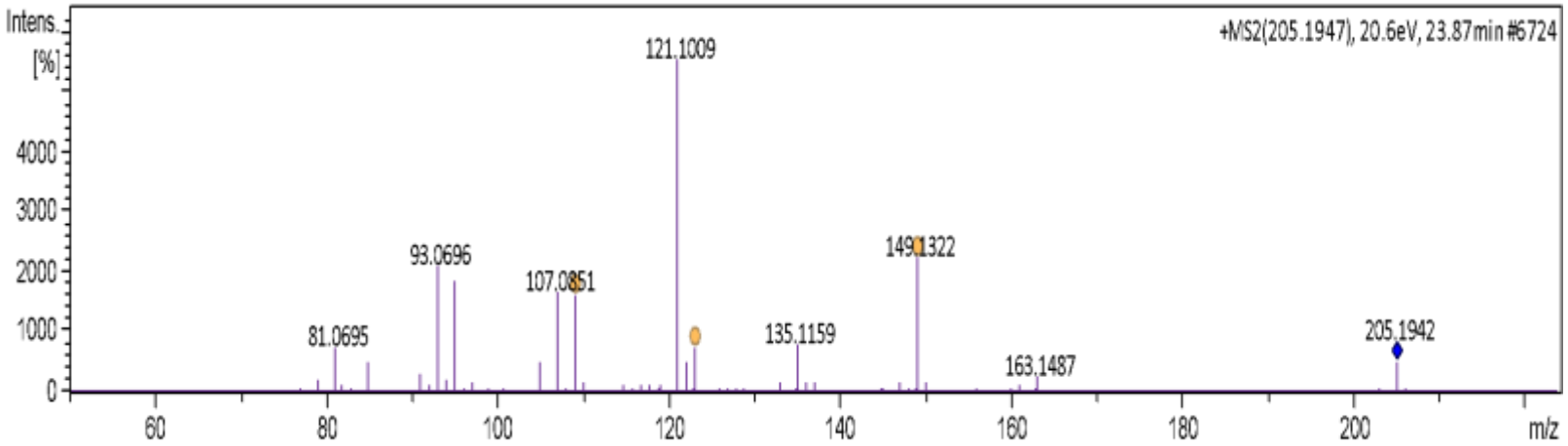

Figure S13- Mass spectra $M+H^+$ 205.1947 ion annotated as germacrene a present in *S. depilis* propolis extracts using data obtained from ultra-high efficiency liquid chromatography analysis coupled to a high resolution spectrometer.

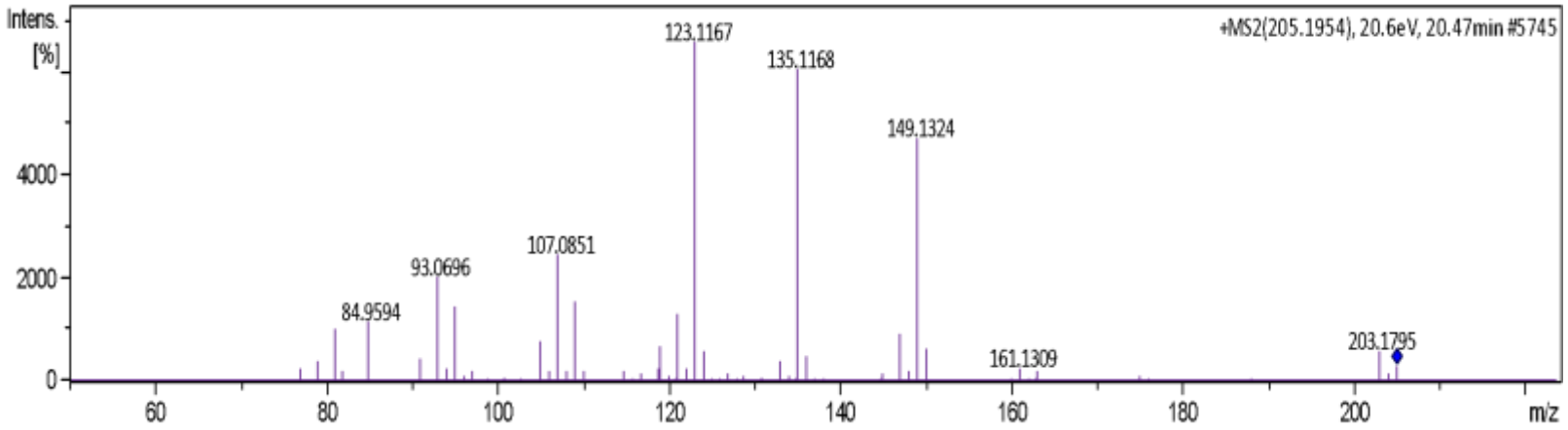

Figure S14- Mass spectra of the $M+H^+$ 205.1954 ion annotated as α-selinene present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

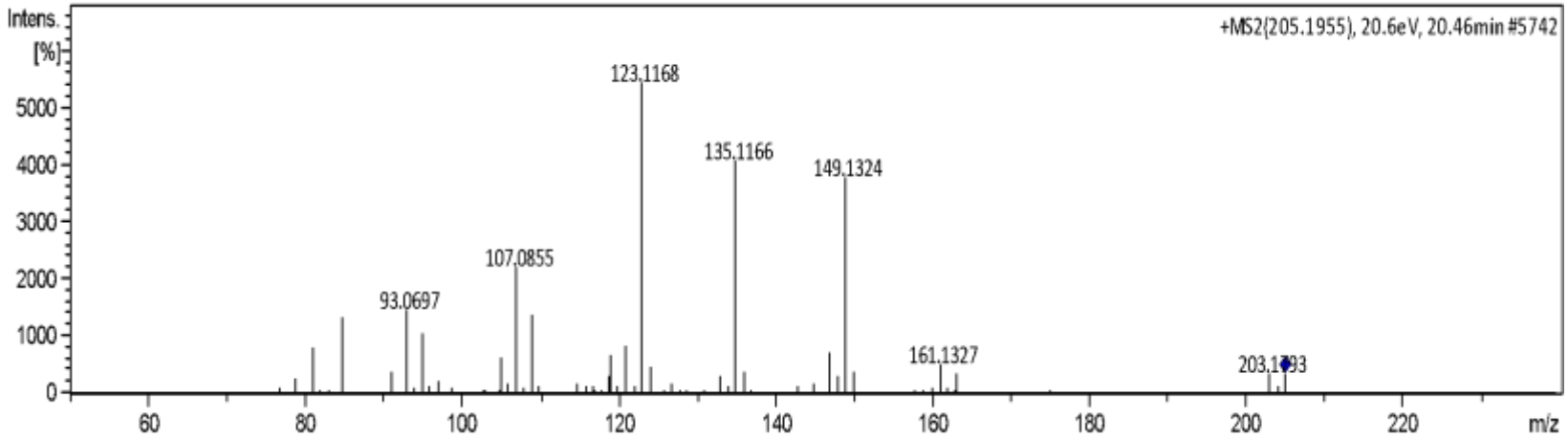

Figure S15- Mass spectra of the $M-H2O+H^+$ 205.1955 ion annotated as α-bisabolol present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

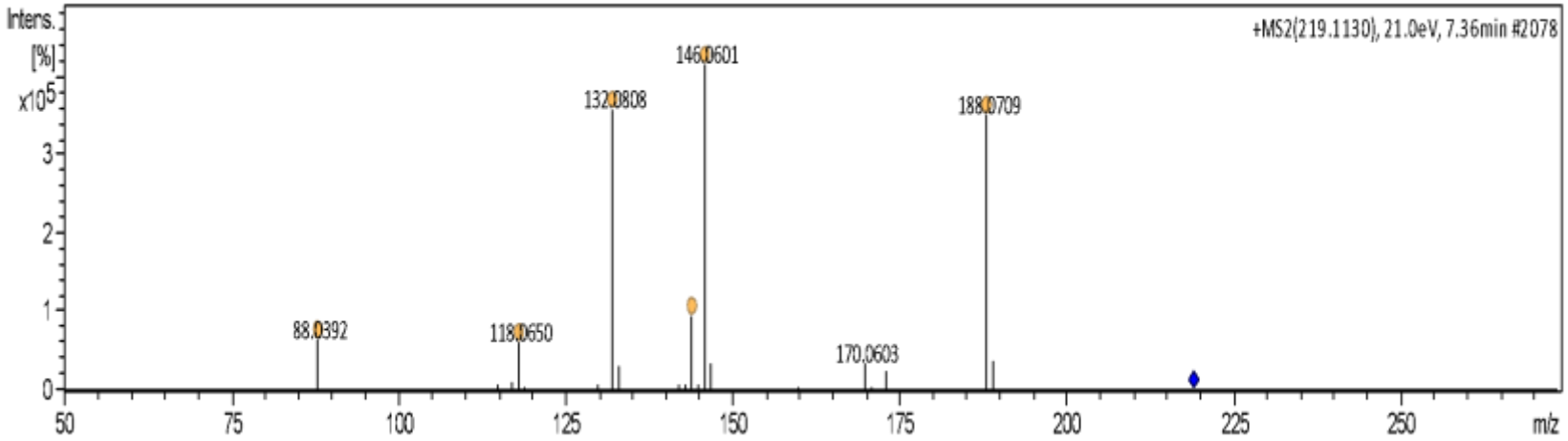

Figure S16- Mass spectra of the $M+H^+$ 219.1130 ion annotated as abrine present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

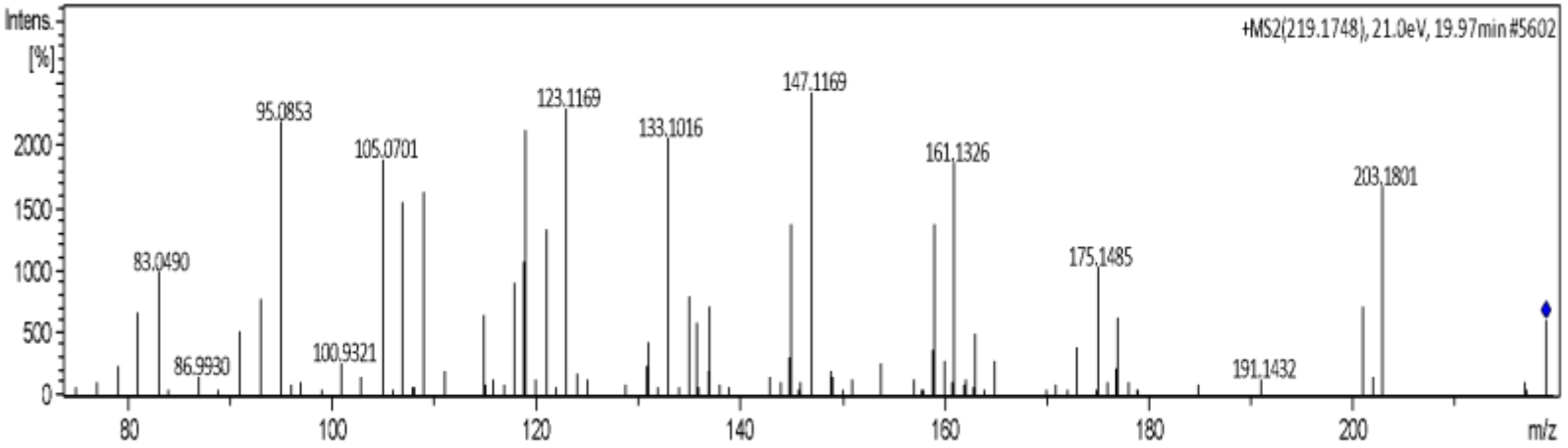

Figure S17- Mass spectra of the $M+H^+$ 219.1748 ion annotated as nootkatone present in the *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

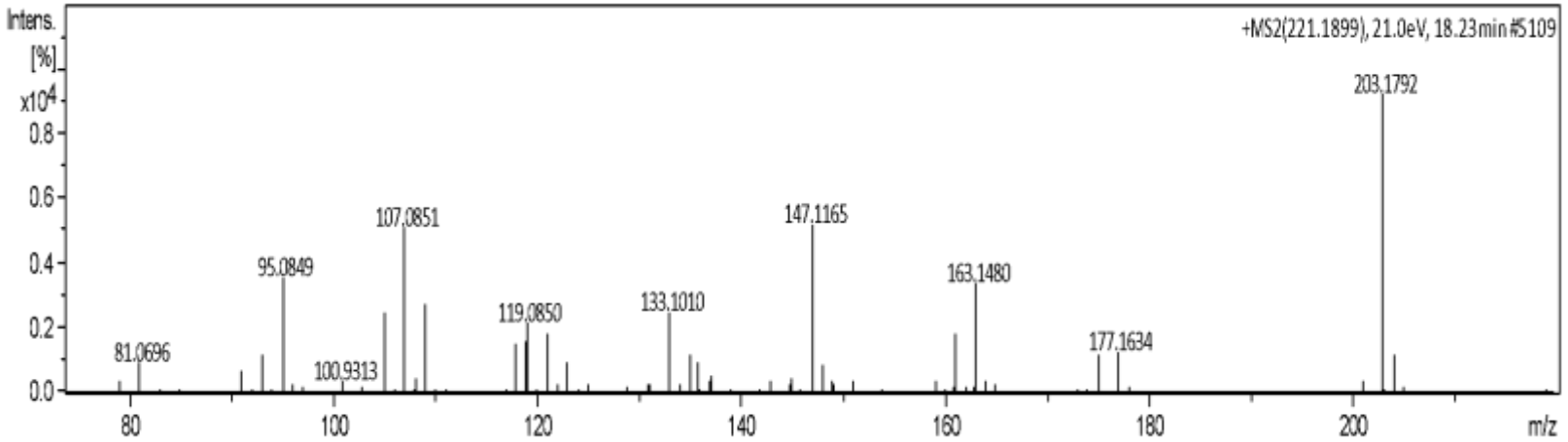

Figure S18- Mass spectra of the $M+H^+$ 221.1899 ion annotated as cyperol present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

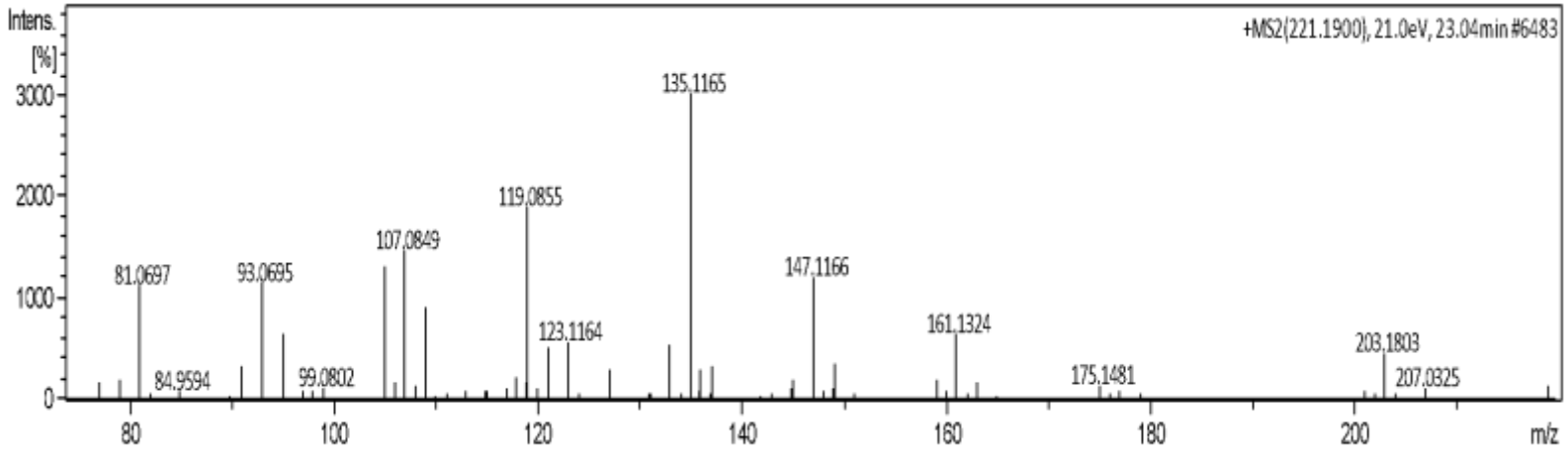

Figure S19- Mass spectra of the $M+H^+$ 221.1990 ion annotated as isocyperol present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

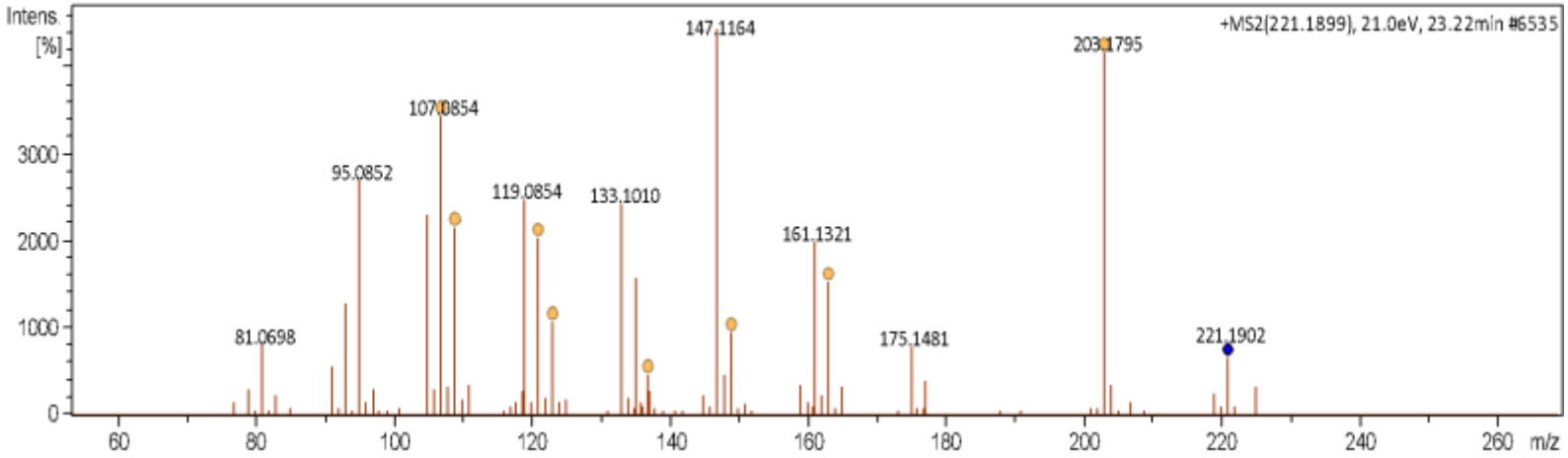

Figure S20- Mass spectra of the $M+H^+$ 221.1899 ion annotated as α-atlantol present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

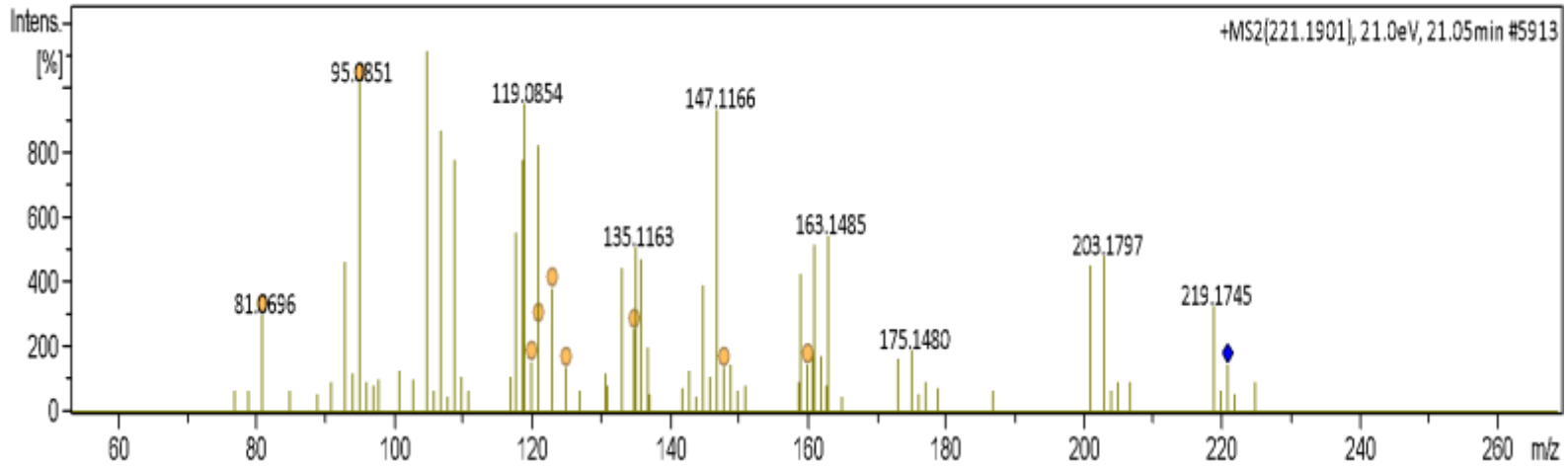

Figure S21- Mass spectra of the $M+H^+$ 221.1901 ion annotated as α-cadinol present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

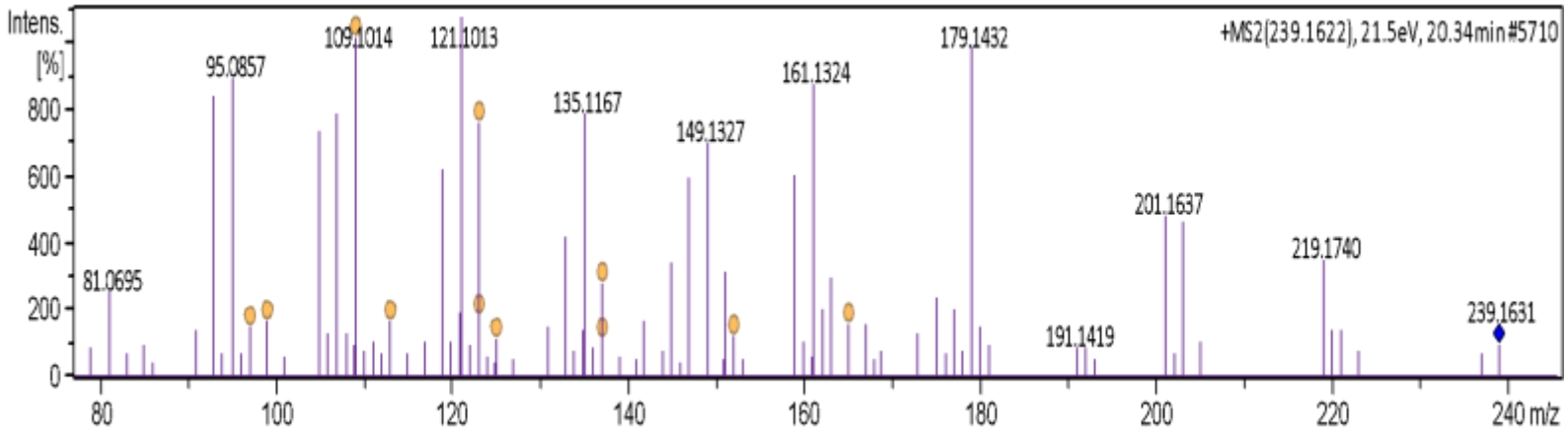

Figure S22- Mass spectra of the $M+H^+$ 239.1622 ion annotated as linalool isovalerate present in the *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

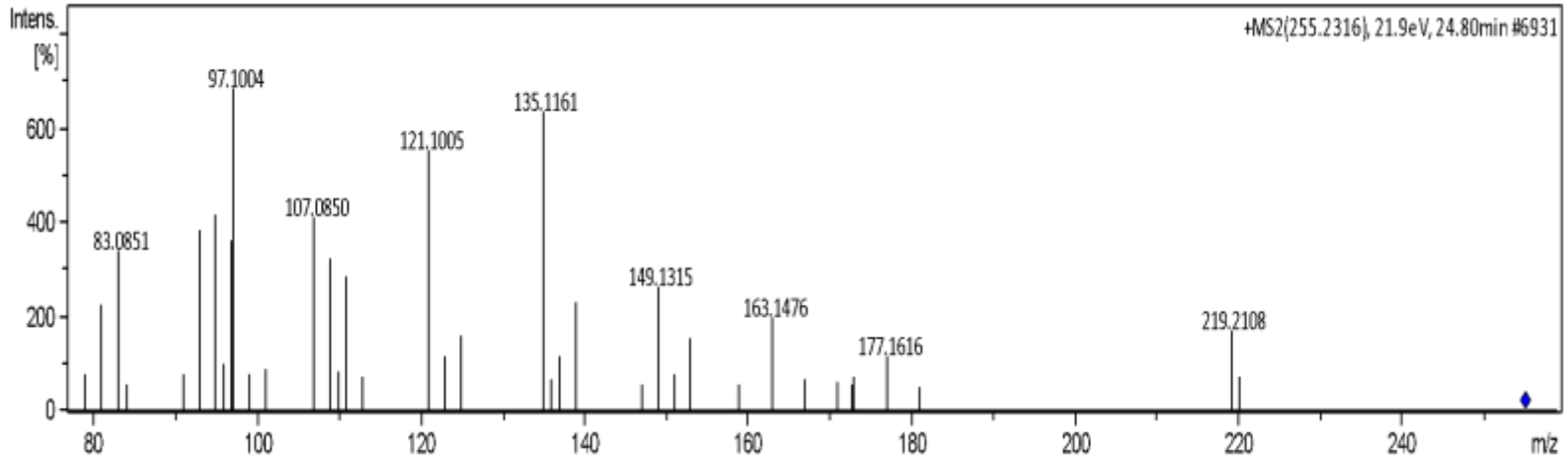

Figure S23- Mass spectra of the $M+H^+$ 255.2316 ion annotated as cis-9-hexadecenoic acid present in the *S. depilis* propolis extracts using data obtained from ultra-high efficiency liquid chromatography analysis coupled to a high resolution spectrometer

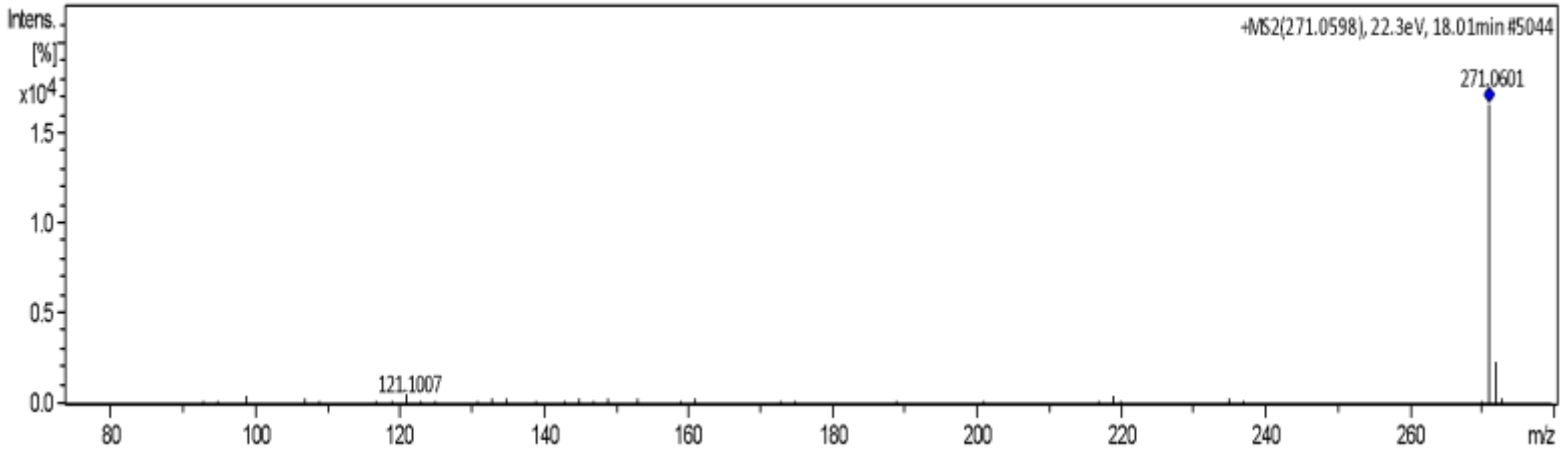

Figure S24- Mass spectra of the $M+H^+$ 271.0598 ion annotated as genistein present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

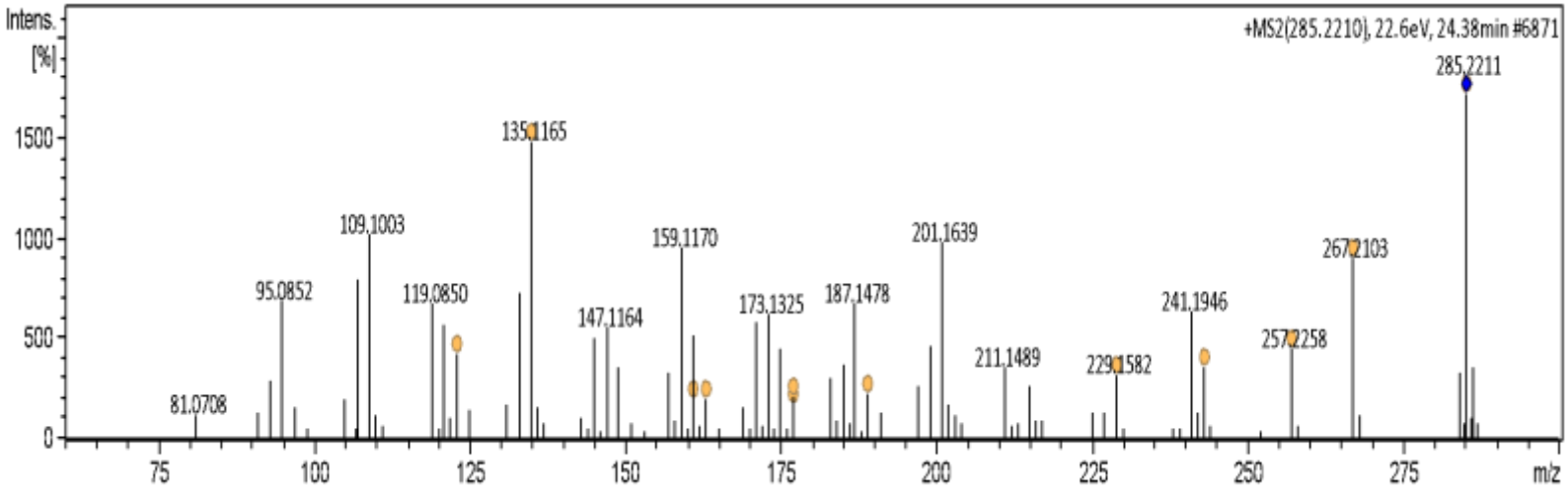

Figure S25- Mass spectra of the $M+H^+$ 285.2210 ion annotated as dehydroabietadienal present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

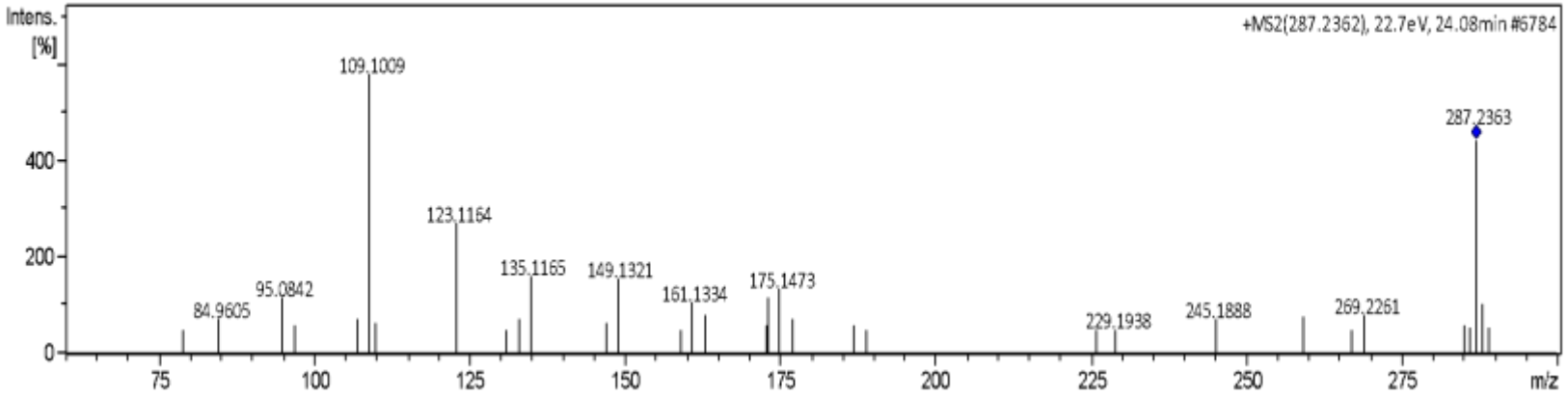

Figure S26- Mass spectra of the M-H2O+H^+ 285.2210 ion annotated as 3-methyl-5-[(1s.8As)-5.5.8 a-trimethyl-2-methylenedecahydro-1-naphthalenyl]-2-pentenoic acid present in the *S. depilis* propolis extracts using data obtained from ultra-high efficiency liquid chromatography analysis coupled to a high resolution spectrometer.

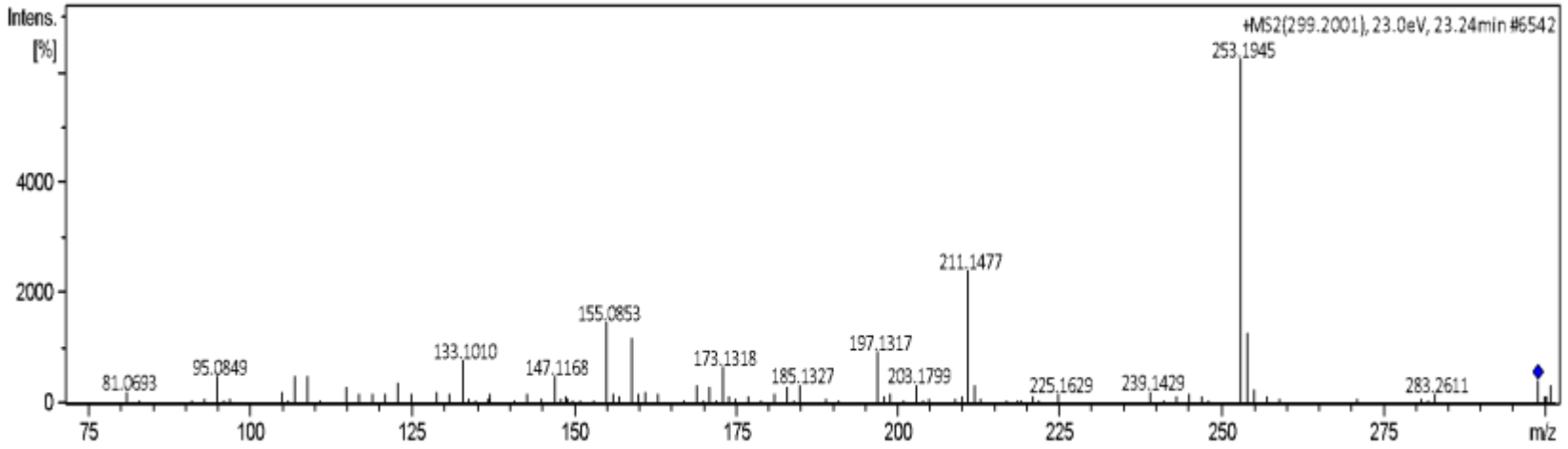

Figure S27- Mass spectra of the M-H2O+H^+ 299.2001 ion annotated as 1-phenanthrenecarboxylic acid, 1,2,3,4,4 a,9,10,10 a-octahydro-9-hydroxy-1,4 a-dimethyl-7-(1-methylethyl) present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

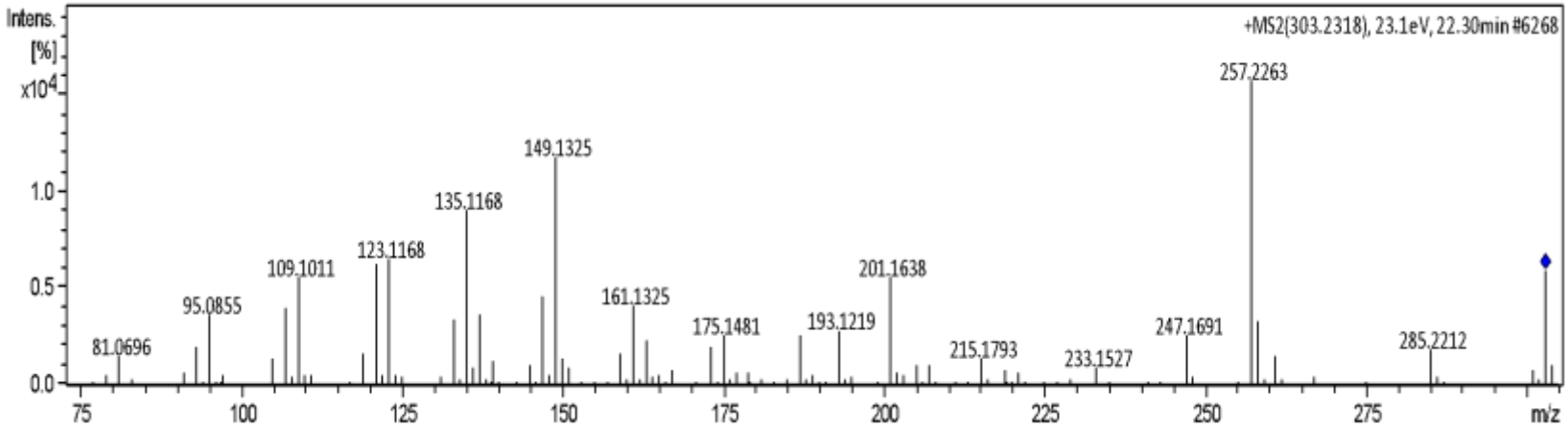

Figure S28- Mass spectra of the $M+H^+$ 303.2318 ion annotated as isopimaric acid present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

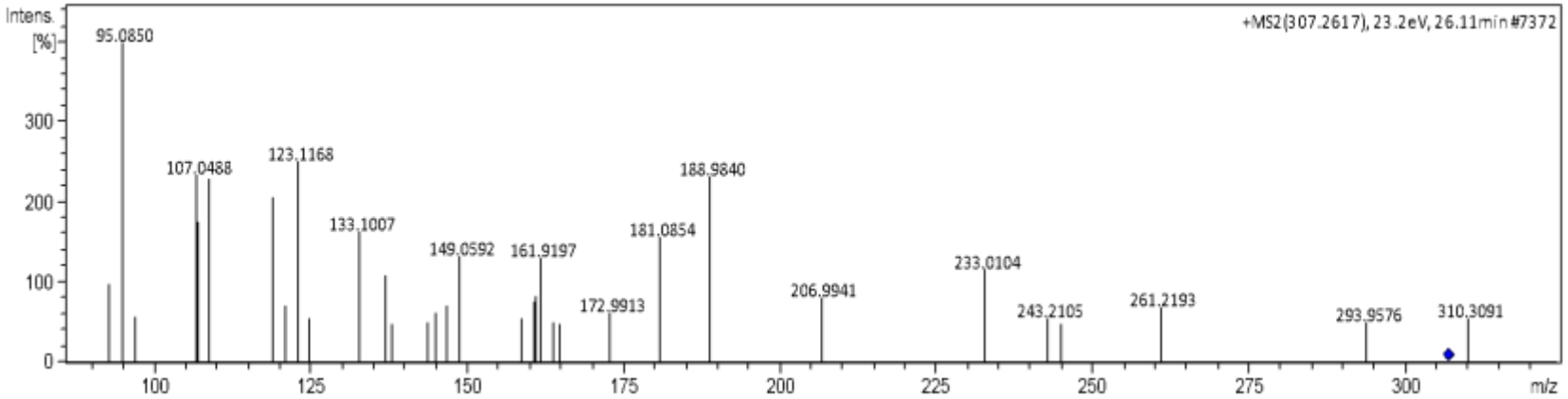

Figure S29- Mass spectra of the $M+H^+$ 307.2617 ion annotated as cis-8,11,14-eicosatrienoic acid present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

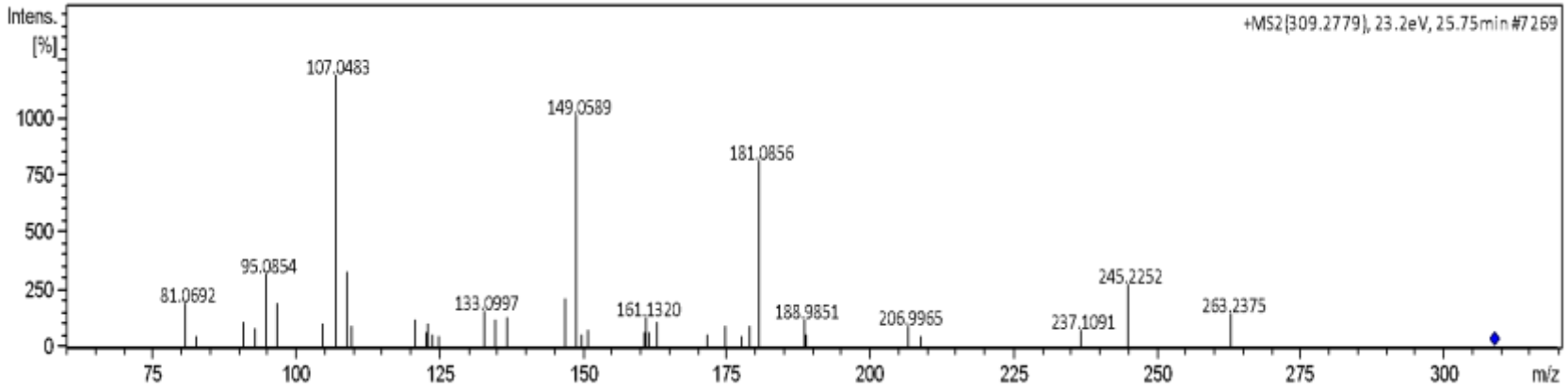

Figure S30- Mass spectra of the $M+H^+$ 309.2779 ion annotated as linoleic acid ethyl ester present in the *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer

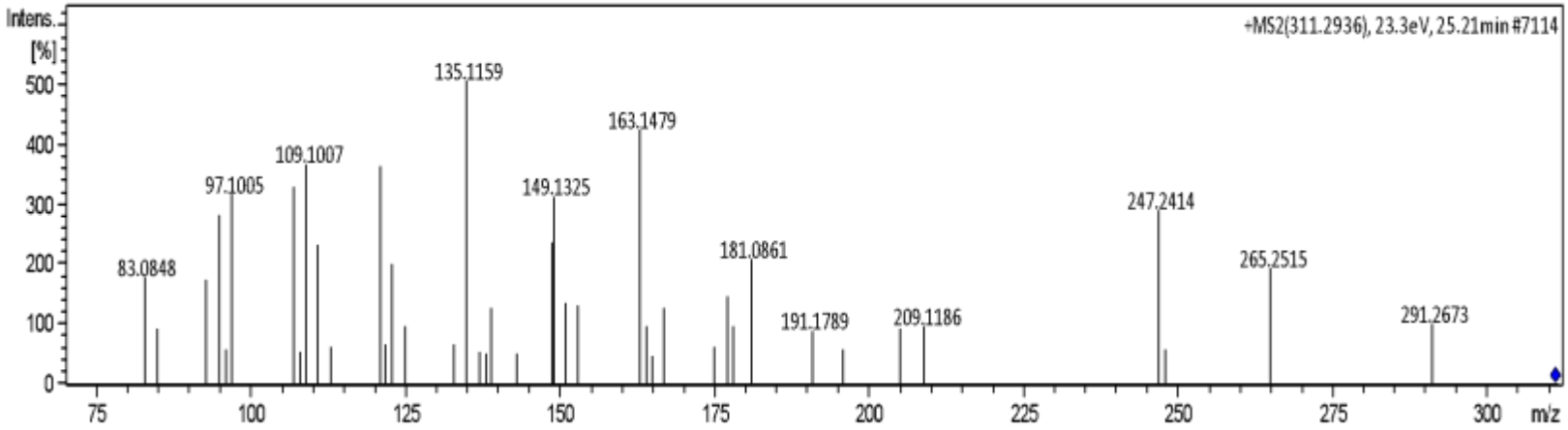

Figure S31- Mass spectra of the $M+H^+$ 311.2950 ion annotated as oleic acid ethyl ester present in the *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

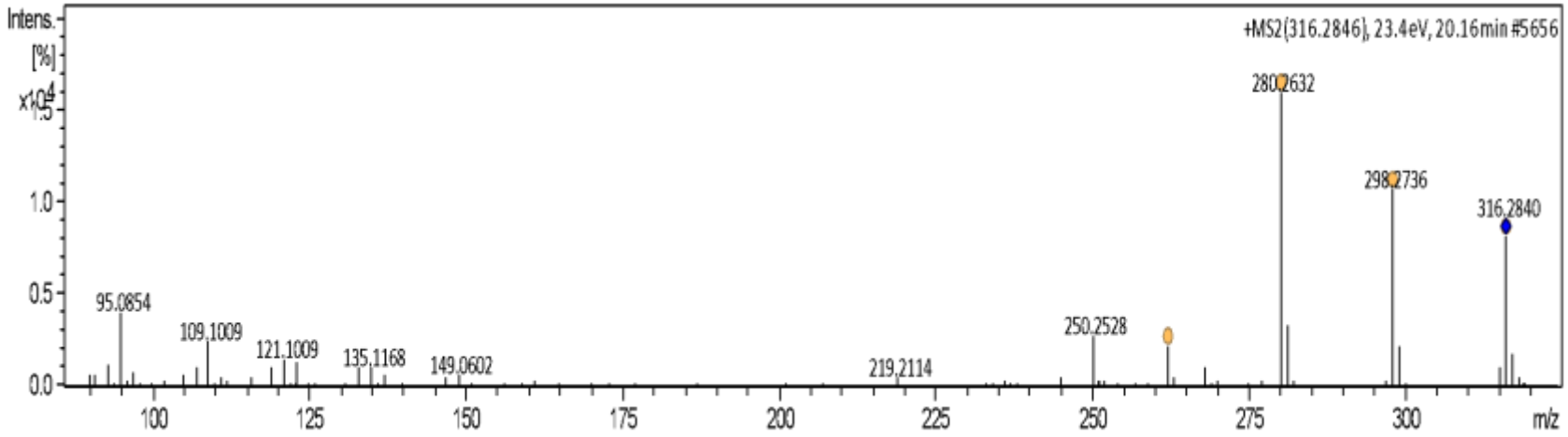

Figure S32- Mass spectra of the $M+H^+$ 316.2846 ion annotated as dehydrophytosphingosine present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

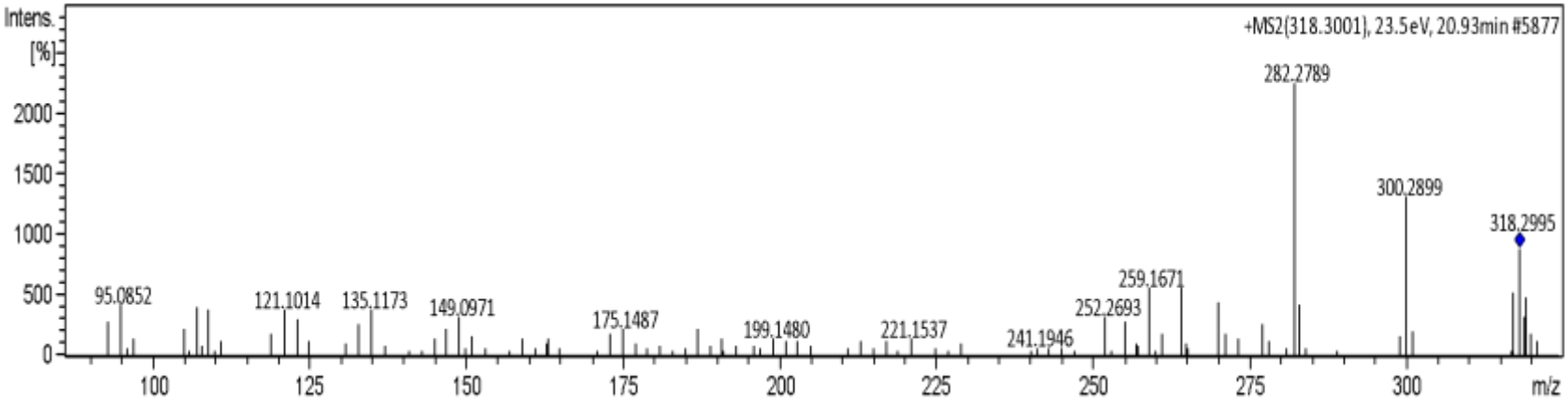

Figure S33- Mass spectra of the $M+H^+$ 316.2846 ion annotated as phytosphingosine present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

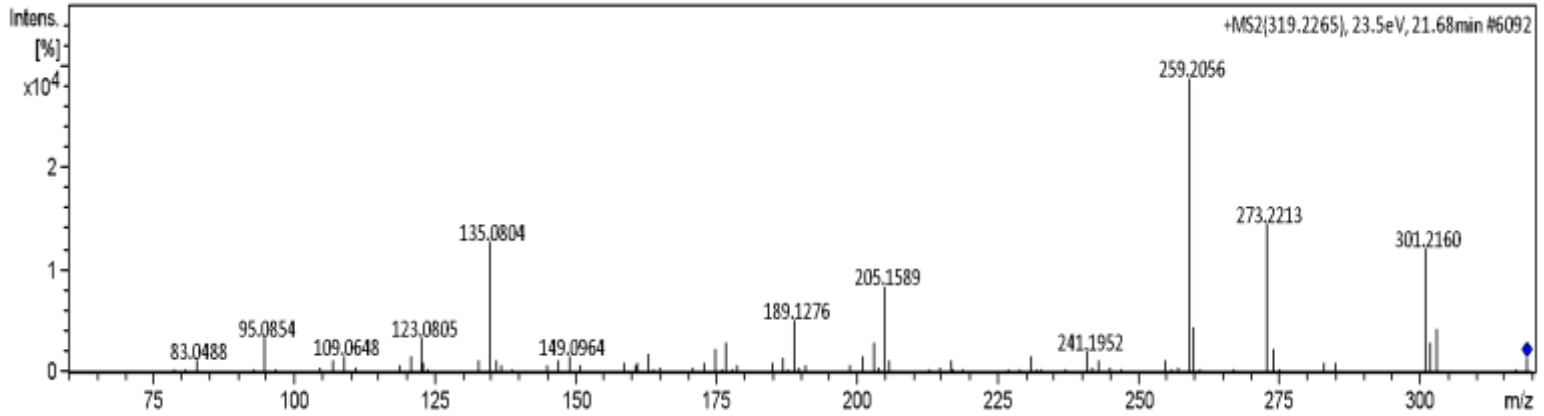

Figure S34- Mass spectra of the $M+H^+$ 319.2265 ion annotated as (*E*)-5-(1,2,4 a,5-tetramethyl-7-oxo-3,4,8,8 a-tetrahydro-2H-naphthalen-1-yl)-3-methylpent-2-enoic acid present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled with a high resolution spectrometer.

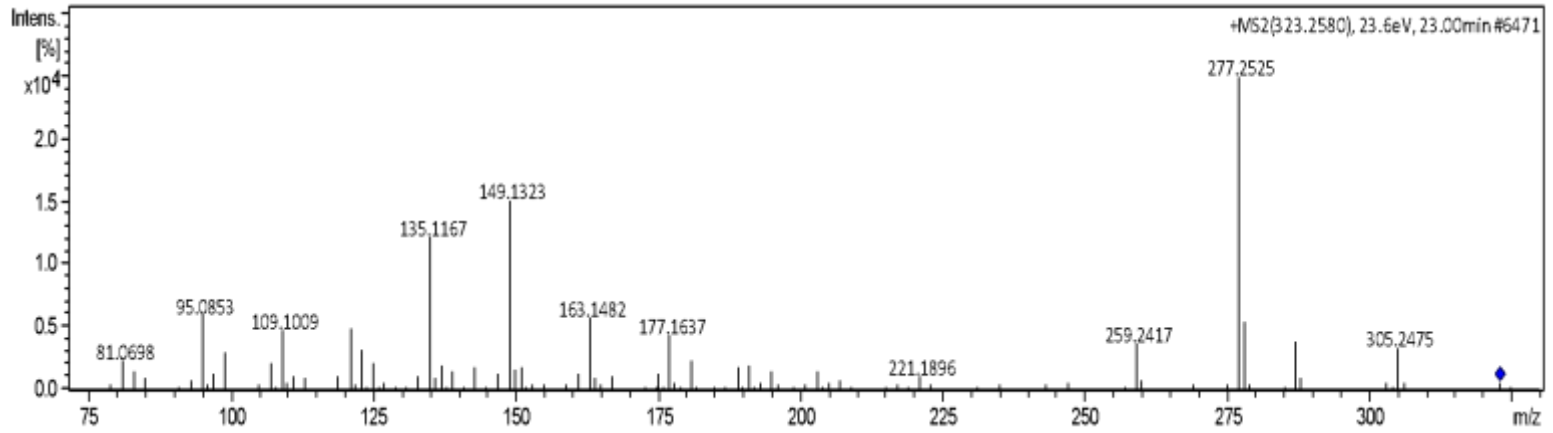

Figure S35- Mass spectra of the $M+H^+$ 323.2580 ion annotated as naphthalenecarboxylic acid, decahydro-5-(5-hydroxy-3-methylpentyl)-1,4 a-dimethyl-6-methylene-, (1R,4As,5R,8aS)- present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

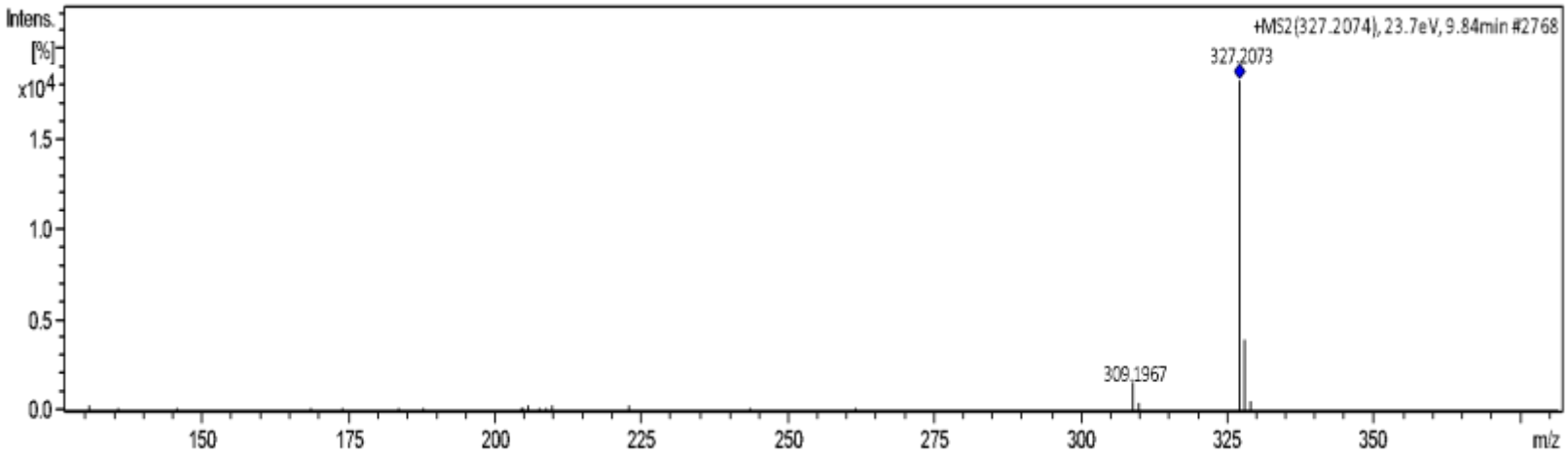

Figure S36- Mass spectra of the $M+H^+$ 327.2074 ion annotated as hydroquinidine present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

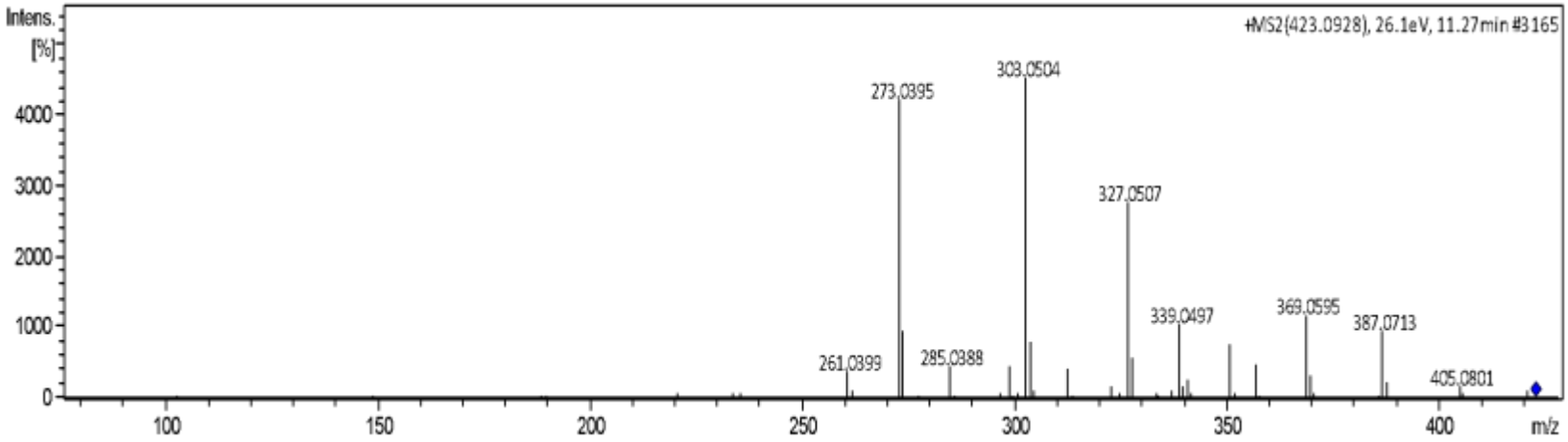

Figure S37- Mass spectra of the $M+H^+$ 423.0928 ion annotated as mangiferin present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

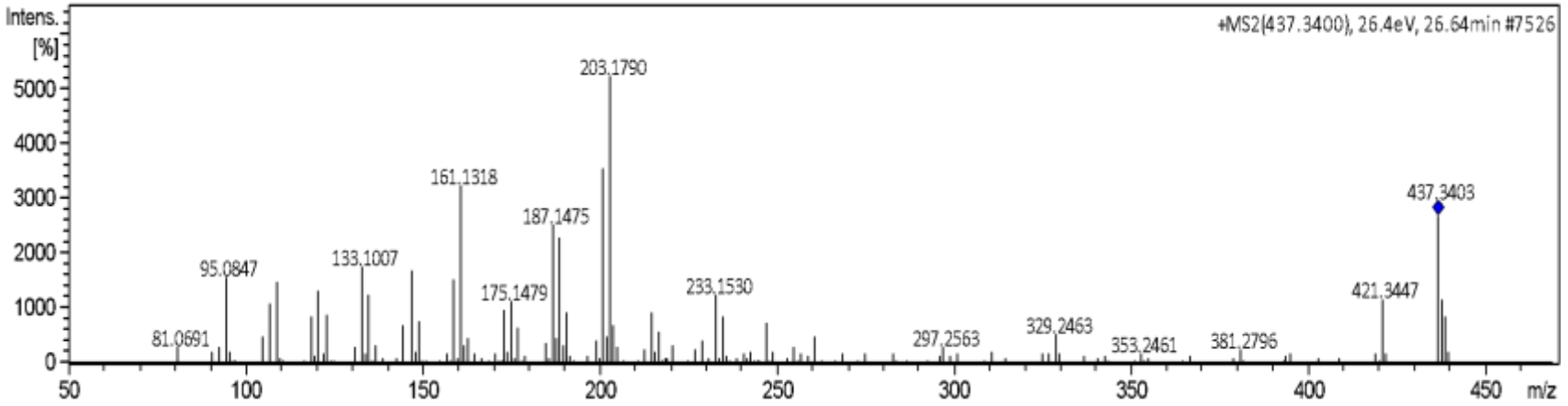

Figure S38- Mass spectra of the $M-H2O+H^+$ 437.3400 ion annotated as 3-hydroxy-11-ursen-28.13-olide present in the *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

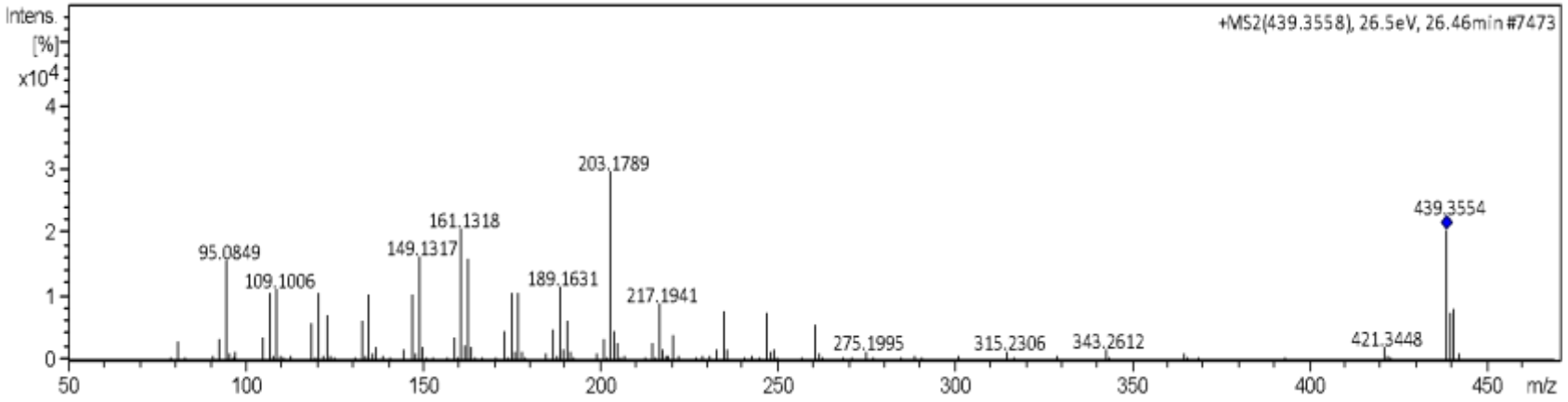

Figure S39- Mass spectra of the $M-H2O+H^+$ 439.3558 ion annotated as oleanolic acid present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

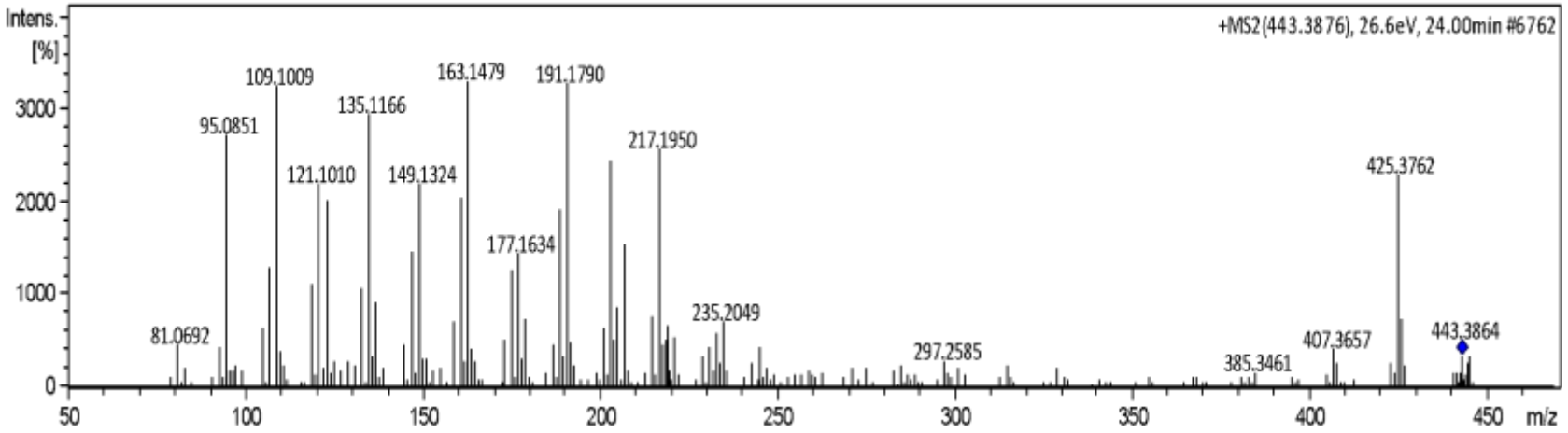

Figure S40- Mass spectra of the $M+H^+$ 443.3876 ion annotated as dipterocarpol present in *S. depilis* propolis extracts using data obtained from analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

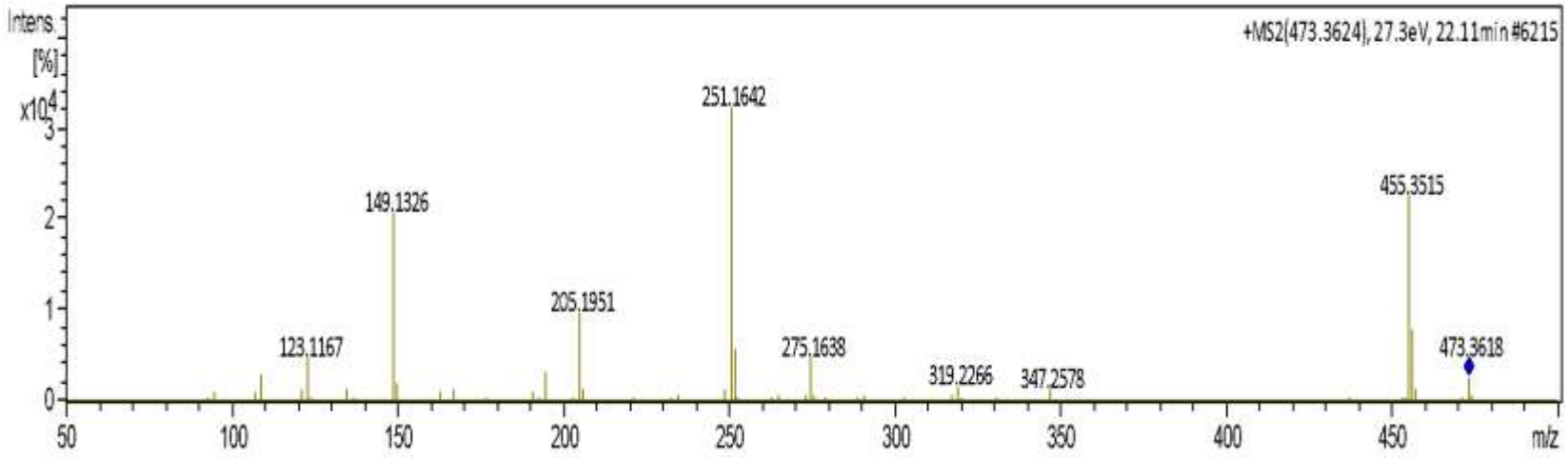

Figure S41- Mass spectra of the $M+H^+$ 473.3624 ion annotated as spinosic acid A present in the *S. depilis* propolis extracts using data obtained from the analysis by ultra-high efficiency liquid chromatography coupled to a high resolution spectrometer.

CONSIDERAÇÕES FINAIS

A revisão de literatura que constitui o capítulo 1 revela o estado da arte das pesquisas com méis de abelhas sem ferrão, a fim de nortear pesquisas futuras para atender expectativas de maiores informações sobre a composição química do mel e propriedades biológicas para agregar valor ao produto e motivar sua comercialização.

No cápitulo 2 os resultados obtidos para o mel in natura e submetido a tratamentos pós-colheita é uma importante contribuição, a fim de verificar as mudanças sofridas após os tratamentos, além do perfil de aceitação em decorrência dessas mudanças.

Já o capítulo 4 mostra que os dados relacionando ao perfil químico da própolis requerem pesquisas mais aprofundadas, entretanto, são aportes iniciais para o conhecimento da diversidade química presente na própolis produzida por *S. depilis*.

De modo geral, o estudo contribuirá para o conhecimento da composição química desses produtos elaborados por essa espécie de abelhas-sem-ferrão, além de propor métodos que auxiliem na comercialização do mel que sobre alterações ao longo do tempo. Os métodos empregados podem orientar na identificação das alterações sofridas nos meis de outras espécies de abelhas-sem-ferrão.