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Chemical analysis of propolis from different regions of Egypt

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Abstract

Propolis samples were extracted from beehives using sharp knife the conventional method in the apiaries located in the Governorates of Sohag, Beheira, Qalubia, Ismailia, and Zagazig. Additionally, two varieties of bee propolis were collected using traps in this investigation as follows: Hand-cut fiber screens with circular holes in a diameter of 1 mm., Fiber mesh sheets (45*35 cm) were put onto the top bar of the combs. Hand - cut polypropylene plastic screens with round holes in diameter of 2mm. Plastic mesh sheets (45*35 cm) were set onto the top bar of the combs inside the honeybee hive presented. Eighty percent methanol was used to extract the samples. Bee propolis was analyzed using LC-MS spectral database, displayed the composition scrape that follows for each Governorate Sohag: ester or ether of tamarixetin-syringic, caffeic-hexose-tartaric, caffeic-quinic acids, gallic-vanillin and acetyl gallate. In Beheira: quinic-hexose, ferulic-hexose, synaptic-pentose or apigenin or one of its isomer malonate, apigenin and vanillic acid. In Qalubia: myricetin, coumaric-hexose-malonate, ferulic-hexose, synaptic pentose or apigenin or one of its isomer malonate. In Ismailia: ferulic-hexose, synaptic-pentose or apigenin or one of its isomer malonate. In Zagazig: Cyanidin, pelargonidin or apigeninidin with benzoic, hydroxyl benzoic. Based on the results of analysis the biological effect of each separated component has been studied.

Introduction

Propolis, or bee glue is a resinous substance secreted from tree stems, buds, or other plant sources that honeybee workers collect and mix with their saliva. Bees protect the colony from unsuitable environmental conditions by sealing the open spaces in the beehive with propolis, moreover grooming brood cells for protection from pathogens, via antifungal and antibacterial properties (Simone-Finstrom and Spivak, 2010).

The composition and nature of propolis depend on environmental conditions and plant sources of resin found in the hive area. It varies considerably from region to region, along with the vegetation (Park *et al.*, 2002). Propolis has many benefits and uses, including limiting the rapid growth of mold and yeast (Ibrahim and Alqurashi, 2022), antioxidant activity (Saleh *et al*., 2023 and Qiao *et al.*, 2023), anti-inflammatory, anti-viral, anti-allergic, anti-cancer and antibacterial effects (Ahangari *et*

al., 2018). Numerous biomedical properties, such as antimicrobial, anti-inflammatory, anti-diabetic, anxiolytic, hepatoprotective, anti-aging and anticonvulsant activities (Seetharaman *et al*., 2017 and Sharaf and Kotb, 2022**).**

Many compounds according to geographic regions were found in the chemical analysis of propolis achieved by different scientists. For example, chlorogenic, quinic, caffeic and dihydrocaffeic acid derivatives (Chen, 2000), rutin, ferulic acid, apigenin, luteolin, quercetin and caffeic acid (Cao *et al.,* 2004), phenolic/aromatic acids (Chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, and cinnamic acid), phenolic aldehyde (vanillin), and flavonoids (pinocembrin, kaempferol, pinobanksin, and apigenin) (Altuntas *et al.,* 2023).

The present work aims to shed light on the chemical analysis of propolis from different regions in Egypt.

Materials and methods

Study Site and Sampling:

Five samples of propolis were collected from apiaries located in Five Governorates which were, Sohag, Beheira, Qalubia, Ismailia and Zagazig. Propolis samples were extracted from beehives using sharp knife using the conventional method, where it was collected by a sharp knife from the top bars of combs, the inner sides of hives and hive entrances. Additionally, two varieties of bee propolis collected traps in this investigation as follows: Hand-cut fiber screens with circular holes in a diameter of 1 mm. Fiber mesh sheets (45*35 cm) were put onto the top bar of the combs presented. Hand-Cut polypropylene plastic screens with round holes in diameter of 2mm. Plastic mesh sheets (45*35 cm) were set onto the top bar of the combs inside the honeybee hive presented. The collected propolis was placed in plastic bags and then kept in refrigerator until use.

Samples were extracted by methanol eighty percent according to the method of Avula *et al.* (2020) then filtered through 0.45 µm micropore membrane before injection (10uL). Samples were injected in volume 5.0 µl and analyzed at a flow rate 0.7 mL/min using Agilent 1200 LC–MS–ESI instrument (Positive mode) with a diode array detector set at 254, 280, 320, 360 and 520 nm. As described by Seetharaman *et al.* (2017). Agilent Zorbax Eclipse Plus C18 column using nitrogen as nebulizing gas was used. The mobil phase used was 1% formic acid (A) and acetonitrile (B); gradient was 0 min 5% B, 1 min 20% B, 6 min 20% B, 8 min 80%B, 18 min 80% B, 19 min 5% B and 20 min 5%. Flow rate 0.7mL/min. Mass scanned in the range m/e 0-1000 at fragmentation energy 70 eV and potential 3.5 kV.

Results and discussion

Chemical compounds detected in bee propolis samples collected from different Governorates during 2020 to 2021:

The identify and quantify the bioactive compounds that are most likely responsible for the antifungal & antibacterial activity observed in samples of propolis collected from different regions in Egypt were determined using Liquid chromatography coupled with mass spectrometry (LC-MS) were summarized in Table (1)and Figures (1-5).

Table (1): The separated phenolic and flavonoid content in bee propolis samples collected from different region in Egypt.

Region	Detected Compound (S)	Rt (min)
Sohag	Ester or ether of Tamarixetin-Syringic	12.06
	Caffeic- Hexose- Tartaric	13.235
	Acetyl gallate	17.440
	Gallic-Vanillin	17.509
	Caffeic- Quinic acids	27.003
Beheira	Apigenin with benzoic acid	12.108
	Ferulic-hexose, synaptic- pentose	12.685
	Quinic-Hexose	13.441
	Vanillic acid or tamarixetin-gallic acid	16.195
Qalubia	Ferulic-hexose, synaptic pentose	13.170
	Apigenin	13.297
	Coumaric-hexose-malonate	28.389
Ismailia	Apigenin	11.999
	Ferulic-hexose, synaptic-pentose	12.708
	Ferulic-hexose, synaptic-pentose	13.170
Zagazig	Pelargonidin	13.56
	Cyanidin or apigeninidin with benzoic	14.59
	dihydroxybenzoic acid respectively	15.51

In sohag Governorate the chemical analysis of propolis (Figure 1) showed the following component according to LC-MS spectral database:

- Ester or ether of Tamarixetin-Syringic (Flavonoids - antioxidant) (Massaro *et al*., 2014).
- Caffeic-Hexose-Tartaric (antioxidant) (Cao *et al*., 2004).
- Caffeic-Quinic acids (antioxidant, antidiabetic, anticancer activity, antimicrobial, antiviral) (Ahangari *et al*., 2018).
- Gallic-Vanillin (Antimicrobial) (Venkata *et al*., 2020).
- Acetyl gallate (Antioxidant) (Altuntas *et al*., 2023).

In Beheira Governorate chemical analysis of propolis (Figure 2) showed the following component according to LC-MS spectral database:

- Quinic-Hexose (antioxidant) (Pecchia *et al*., 2011).
- Ferulic-hexose, synaptic-pentose or apigenin or one of its isomer malonate (Antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, and antiproliferative activities.) (Sahnoun *et al*., 2018).
- Apigenin or one of its isomers (baicalein, galangin, genistein) with benzoic acid, or chrysin or diadzin with hydroxybenzoic acid (antioxidant, anticarcinogenic, anti-inflammatory) (Al-huqail *et al*., 2019).
- Vanillic acid or tamarixetin-gallic acid (Anti-inflammatory) (Alshehri *et al*., 2021).

In Qalubia Governorate chemical analysis of propolis (Figure 3 A and B) showed the following component according to LC-MS spectral database:

- Coumaric-hexose-malonate (Antioxidant) (Al-huqail *et al*., 2019).
- Ferulic-hexose, synaptic pentose or apigenin or one of its isomer malonate (Antioxidant,

anticarcinogenic, anti-inflammatory) Sharaf and Kotb (2022).

- A pigenin (Anti-fungal) (Sahnoun *et al.* , 2018).

In Ismailia Governorate chemical analysis of propolis (Figure 4 A and B) showed the following component according to LC-MS spectral database:

- Ferulic-hexose, synaptic-pentose or apigenin or one of its isomer malonate (Antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, and antiproliferative activities) (Sharaf and Kotb, 2022).

In Zagazig Governorate chemical analysis of propolis (Figure 5) showed the following component according to LC-MS spectral database:

- Cyanidin, pelargonidin or apigeninidin with benzoic, hydroxyl benzoic or dihydroxybenzoic acid respectively (Antioxidant, anti-inflammatory) (Omar *et al*., 2018).
- Pelargonidin or apigeninidin (Anti-fungal) (Sahnoun *et al.*, 2018).

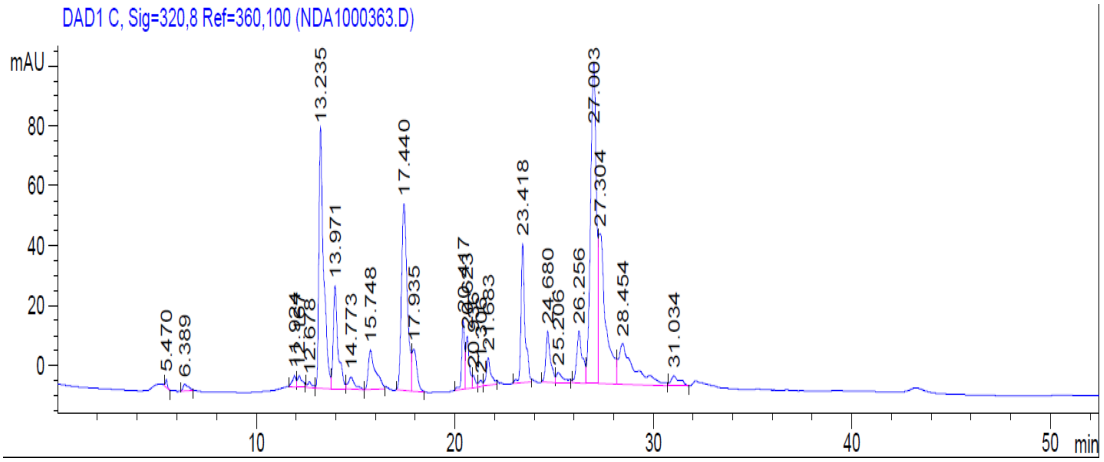


Figure (1): Chromatogram of the separated compounds of propolis samples collected from the apiary located in Sohag Governorate.

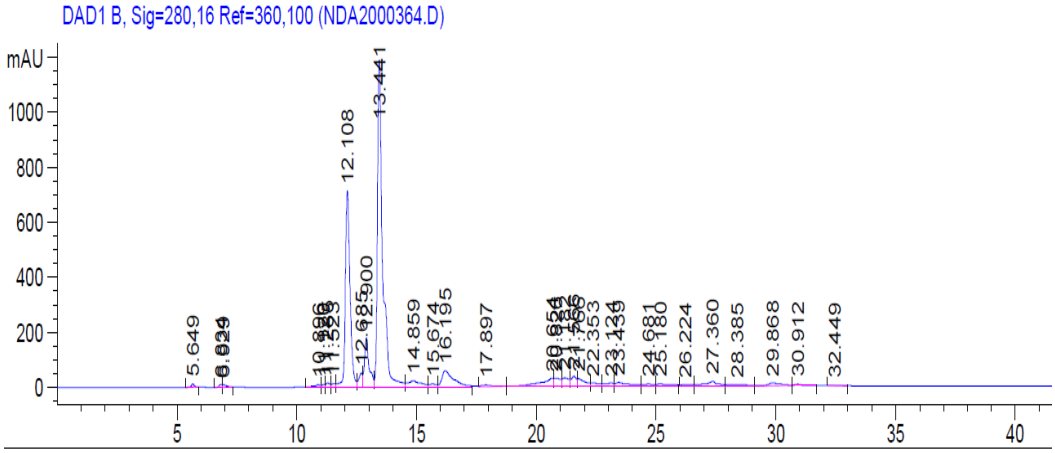
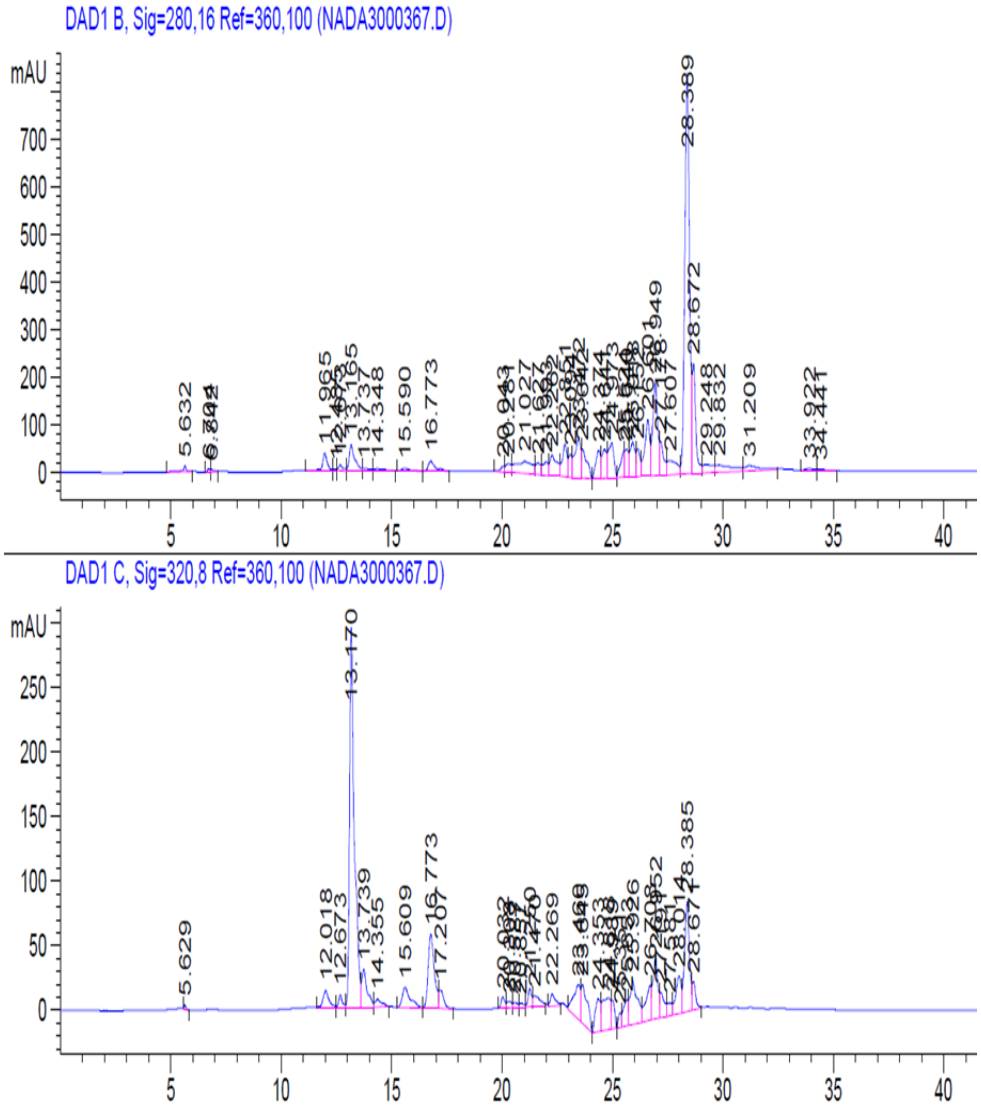


Figure (2): Chromatogram of the separated compounds of propolis samples collected from the apiary located in Beheira Governorate.



(A)

(B)

Figure (3): Chromatogram of the separated compounds extracted from the propolis samples collected from the apiary located in Qalubia governorate. Where, (A) Coumaric-hexose-malonate (Retention peaks were seen at 28.389) and (B) Ferulic-hexose, synaptic pentose -apigenin (retention peaks were seen at 13.170, 13.297).

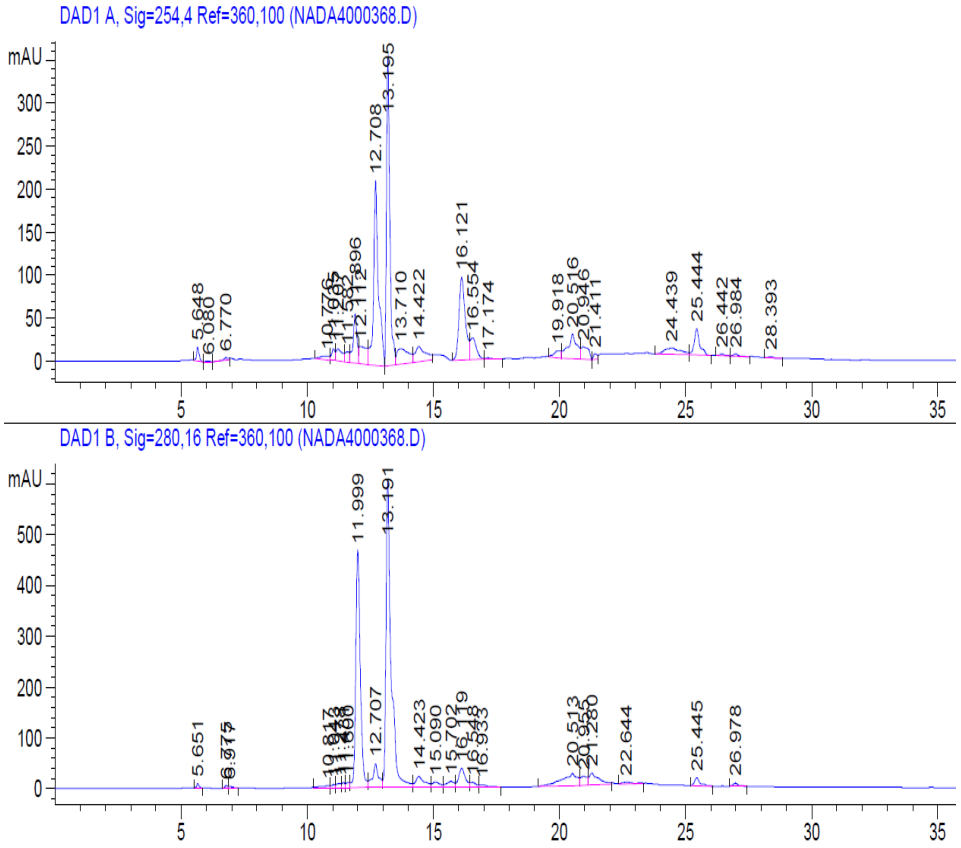


Figure (4): Chromatogram of the separated compounds extracted from the propolis samples selected from the apiary located in Ismailia Governorate. Where, (A) Ferulic-hexose, synaptic-pentose (Retention peaks were seen at 12.708) and (B) Ferulic-hexose, synaptic-pentose-apigenin (Retention peaks were seen at 13.170, 11.999).

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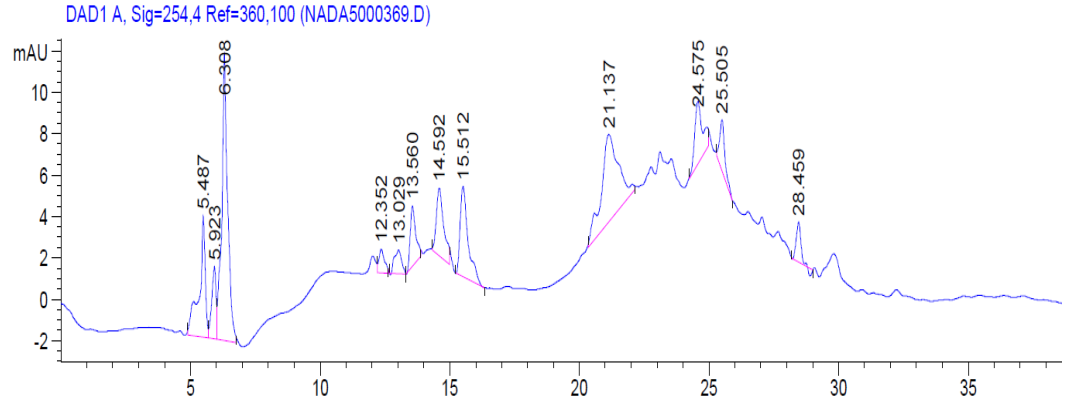

Figure (5): Chromatogram of the separated compounds of propolis samples selected from the apiary located in Zagazig Governorate.

The elements found in the five samples per region show a pattern that can be identified as follows: Such differentiation of the obtained chemical analysis could be due to plant source and environmental variation of different governorates.

There are many authors mentioned that they used different phenolic compounds such as, flavonoids, triterpenoid saponins and quinic acid for major flavonoids rutin,

quercetin, myricetin, kaempferol), gallic and vanillic acid Pecchia *et al*. (2011) and Dimcheva *et al*. (2019). Ibrahim and Alqurashi (2022) found that their study aimed to identify the phenolic compounds present in propolis demonstrated propolis an antifungal activity, pH, color, and sensory analysis of creamy cheese with thyme. Ibrahim and Alqurashi (2022) identified the phenolic compounds present in propolis and evaluated their antifungal activity. Sharaf and Kotb (2022) reported that, the biological activity of the antimicrobial, antioxidant and anti-inflammatory.

The present review summarizes information concerning the biological activities of some compounds isolated from *F. vulgaris* in addition to their chemical structures apigenin and phenolic acids. Chen (2000) used different antioxidants against invasive fungal infection, a major cause of morbidity and mortality inimmuno-compromised hosts, which is of importance and is significantly increasing in incidence in recent years. Chromogenic, quinic, caffeic and dihydrocaffeic acid derivatives. Massaro *et al.* (2014) indicated known C-methylated flavanones namely, 1 (2S)-cryptostrobin, its regioisomer 2 (2S)- stroboponin, 3 (2S)-cryptostrobin7-methyl ether, and 6 (2S)-desmethoxymatteucinol, and known flavanones 4 (2S)- pinostrobin and5 (2S)-pinocembrin as markers for *C. torelliana* fruit resins and one propolis type and used different antibacterial such as, working on isolation, structural elucidation and antibacterial testing of flavanones of *C. torelliana* fruit resins that are incorporated into stingless bee propolis C-methylated flavanones.

Omar *et al*. (2018) the study was to evaluate in-vitro growth inhibition activity of benzoic acid against some pathogenic fungi benzoic acid. Venkata *et al.* (2020) the antifungal efficacy of Van (Vaniline) demonstrated by the enhanced survival of *C. elegans* with Candida infection. Different antifungal such as, Chrysin (5,7-dihydroxy flavone, ChR) is a flavone of plant origin, possessing numerous biomedical properties, such as antimicrobial, anti-inflammatory, anti-diabetic, anxiolytic, hepatoprotective, anti- aging and anticonvulsant activities were studied Seetharaman *et al.* (2017). Sahnoun *et al*. (2018) showed an inhibitory effect of apigenin towards Human and *Aspergillus oryzae* $2 a-amylases. Apigenin inhibition towards Human and *A. oryzae* aamylase activities was observed to be competitive. El-Nagar *et al.* (2020) investigated the in vitro antifungal properties of gallic acid and two of its derivatives (syringic and pyrogallic acids) against *A. solani* during 2019 and 2020 seasons. Taheri *et al.* (2020) said that Several flavonoids have been recognized as nutraceuticals, and myricetin is a good example, Myricetin is commonly found in plants and their antimicrobial and antioxidant activities is well demonstrated. Alshehri *et al*. (2021) followed by the presence of tannins. In addition, different parts have revealed the existence of steroids, flavonoids, Tamarix, cardiac glycosides, and byproducts of gallic acid and ellagic acid. Nandekar and Gurnule (2021) The copoylmer characterization was done with IR and 1H NMR Spectra. The antibacterial and antifungal screening of copolymer p-HBSF-I was done by using a well-known agar diffusion method. The bacterial strains used were *staphylococcus aures*, *Bacillus subtillus*, *Escherchia coli*, *Salmonella typhi* and the fungal strains used were *Aspergillus niger*, *Candida albicans*.

The present study includes the antimicrobial investigation of copolymer resins to know the effectivity of semicarbazide group.

Ester or ether which was discovered by Massaro *et al.* (2014).and was exclusively present in our Zagazig sample. identified constituents of propolis were

phenolic/aromatic acids (chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, and trans-cinnamic acid), phenolic aldehyde (Vanillin), and flavonoids (pinocembrin, kaempferol, pinobanksin, and apigenin). Altuntas *et al*. (2023), Saleh *et al*. (2023) bee propolis is one of the most common natural extracts and has gained significant interest in biomedicine due to its high content of phenolic acids and flavonoids, which are responsible for the antioxidant activity of natural products. Additionally, Stojko *et al.* (2015) discovered that quinic acid was present in our Sohag and Beheira samples. Chrysin was detected in both our Beheira and Ismailia samples which is consistent with what. Seetharaman *et al.* (2017) showed that Chrysin (5,7-dihydroxy flavone, ChR) is a flavone of plant origin, thin layer chromatography and high-performance liquid chromatography profiles of fungal extracts showed Rf values and retention times that closely match those of standard chrysin (ChR). Further, the production of fungal chrysin (FChR) was confirmed through UV-vis spectroscopy, FT-IR, LC-ESI-MS, and 1H1NMR analysis, according to Omar *et al.* (2018) evaluated in vitro growth inhibition activity of benzoic acid against some pathogenic fungi. The pathogens. used in the test were seven species of *Fusarium* (*F. moniliforme, F. oxysporium, F. solani, F. graminearum, F. sambucinum, F.equisetum and F.semitectum), Rhizoctenia solani* and *Verticillium dahliae* and Al-Huqail *et al.* (2019) found that phenolic and flavonoid compounds in the water extract were analyzed using HPLC: benzoic acid, caffeine, and o-coumaric acid were the most abundant phenolic compounds; while the flavonoid compounds naringenin, quercetin, and kaempferol were identified compared with the standard flavonoid compounds.

Additionally, it was noted that our Sohag and Beheira samples contained Vanillic acid, by Dimcheva *et al*. (2019). Additionally, reported that high-performance liquid chromatography with diode-array detection (HPLCDAD method was developed for simultaneous determination of 9 natural substances common in plants: three major catechins ((-) - epicatechingallate, (-) - catechin, (-) - epigallocatechin), four major flavonoids (rutin, quercetin, myricetin, kaempferol), gallic and vanillic acid. Related derivatives (Chlorogenic, quinic, caffeic and dihydrocaffeic acid derivatives) were later designed to mimic the backbone of echinocandins. Further studies have been carried out on the optimization of synthesis and modifications of a caffeic acid derivative according to Chen (2000).

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