

English Summary:

Propolis is the most important 'chemical weapon' of bees against pathogenic microorganisms, (sometimes also referred to 'bee glue'), Bee glue's chemical composition depends on the specificity of the local flora at the site of collection and thus on the geographic and climatic characteristic of this site. This fact results in the striking diversity of propolis chemical composition; however it composed mainly of 50% resin (composed of flavonoids and related phenolic acids), 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds. Numerous studies, carried out with the combined efforts of phytochemists and pharmacologists, led in recent years to the idea that different propolis samples could be completely different in their chemistry and biological activity. Propolis has been used as a remedy by humans since ancient times. It is still one of the most frequently used remedies for treatment of wounds and burns, sore throat, stomach ulcer, etc. For this reason propolis has become the subject of intense pharmacological and chemical investigations for the last 30 years.

The present work includes: investigation of the volatile constituents, isolation and identification of constituent, as well as evaluation of certain biological activities of the PEF.

1. Investigation; of the volatile constituents:

GC/MS analysis of the volatile constituents of Egyptian propolis revealed the identification of 31 compounds constituting 80% of the total volatiles of PEF. Twelve oxygenated compounds were identified and attributed mainly to aromatic compounds (72.58%) , terpenoids compounds (2.43%) and three unsaturated HC compounds (14.54%) . Nineteen non oxygenated compounds mainly attributed to aromatics compounds (34.05%), saturated hydrocarbons (one compound = 1.00) constituted followed by unsaturated HC (one compound = 0.95%), and then sesquiterpene terpenoids (one compound = 2.43%).

2. Investigation of compounds:

2.1. Isolation and identification of compounds:

Propolis (1 Kg.) was cut into small pieces and extracted with distilled water (2.5L x 2) each for 2 hours at 85 °C the residue was fractionated with 70% ethanol (2L x 2) under the reflux conditions for 2 h. The residue was again fractionated with ethanol absolute (2L.x2) under the reflux conditions for 2 h which gave the ethanol absolute fraction (PEF) = (50 g) after evaporation.

PEF (50 g) was subjected to Sephadex LH-20 column chromatography (100x10 cm). Stepwise gradient elution was carried out using a solvent system of decreasing polarity starting

with 100% distilled water, water–methanol and then methanol–methylene chloride. Fractions of 500 ml were collected and investigated by TLC (silica gel DF₂₄₅ Merck) using different spraying reagents resulted in four main fractions. Each fraction was rechromatographed on silica gel columns. The isolated compounds were further purified by recrystallization.

Structure elucidation and identification of the isolated compounds were carried out using spectroscopic analysis comprised UV, ¹H-NMR, ¹³C-NMR, and EI/MS methods, and comparing the obtained data with that available in the current literature.

2.1. 1. Isolated flavonoids:

Three known flavonoid were isolated and identified from PEF, one flavone (chrysin) with two mixture one of them contains (chrysin and acacetin) and the other one contains (chrysin , chloro- chrysin).

2.1. 2. Isolated terpenoids:

Four known terpenoid were isolated and identified from PEF comprising α -amyrin acetate, 3 β - Cycloartenol, 3 β Cycloartenol-26-oic acid and mixture of α,β - Cycloartenol

2.1. 3. Isolated compounds still under investigation:

One unknown compound was isolated.

3. Biological study:

Biological studies were carried out on PEF as follows:

3.1. *In – vitro*:

3.1.1. Antioxidant activity:

3.1.1.1. Chemically using DPPH radical scavenging assay:

Radical scavenging activity of PEF and isolated compounds against stable DPPH was determined Spectrophotometrically, Mix A (chrysin and acacetin) was the most effective DPPH radical scavenger with the inhibition of 52.66 %, followed by Compound **5** (chrysin) with the inhibition of 45.56 % and PEF with the inhibition of 84.02 %, Compounds **1, 2, 3, 4** showed very weak or no activity.

3.1.1.2. Enzymatic method by determining the superoxide anion radical scavenging activity:-

Radical scavenging activity of PEF and isolated compounds against the superoxide anion radical scavenging activity by generating superoxide anion free radical in xanthine–xanthine oxidase (X–XOD) system was measured. Compound **2** exhibited the highest XOD scavenging activity within the conc. of 50 µg / ml (95.16 % inhibition), followed by Compound **1** and Mix A

with inhibition of 94.08 % then Compound **5** with inhibition of 92.47%. Compounds **4,3**, Mix B showed inhibition of 89.78 %, 78.49 %, 89.24 % respectively. PEF extract inhibitions of 88.70% and compound **6** cannot be detected because of their colour.