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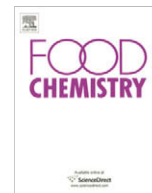

# Bioactivity of Greek honey extracts on breast cancer (MCF-7), prostate cancer (PC-3) and endometrial cancer (Ishikawa) cells: Profile analysis of extracts

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ABSTRACT

Historically, honey has been important in Greek culture. The chemical composition and the potential of Greek honey extracts (thyme, pine and fir honey) to influence the oestrogenic activity and the cell viability of breast (MCF-7), endometrial (Ishikawa) and prostate (PC-3) cancer cells were investigated. All honeys contained total phenolics, phenolic acids and hydroxymethylfurfural, the levels being highest in thyme honey. Sugars and volatile compounds, but not fatty acids, were detected in all honey extracts. Thyme, pine and fir honey showed both antioestrogenic and a weak oestrogenic effect at low and high concentration, respectively, in MCF-7 cells. Thyme honey reduced the viability of Ishikawa and PC-3 cells, whereas fir honey stimulated the viability of MCF-7 cells. In conclusion, Greek honeys are rich in phenolic compounds, they modulate oestrogenic activity whereas a thyme honey-enriched diet may prevent cancer-related processes in breast, prostate and endometrial cancer cells.

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## 1. Introduction

The benefits of bee honey in human health have long been recognised. Athenaeus, the Greek philosopher and author of "*The deipnosophists*", reported that Democritus (500 BC) used honey in his daily diet for longevity and fertility. Democritus, Hippocrates and Discorides all considered honey as an important agent for the strengthening of the body and the promotion of health (Rüdiger, 1977), and, Plato's concepts of a healthy diet consisted of cereals, legumes, fruits, milk, honey and fish (Skiadas & Lascaratos, 2001).

This natural product exhibits antioxidant, chemopreventive, antiatherogenic, immunoregulatory, antimicrobial and wound healing properties (Al-Waili & Haq, 2004; French, Cooper, & Molan, 2005; Gheldof & Engeseth, 2002; Mabrouk et al., 2002; Molan, 2006; Schramm et al., 2003; Swellam et al., 2003; Tonks, Cooper, Price, Olan, & Jones, 2001). Various signalling pathways, including stimulation of TNF-alpha (tumour necrosis factor-alpha) release, inhibition of cell proliferation, induction of apoptosis and cell cycle arrest, as well as inhibition of lipoprotein oxidation, mediate the beneficial effects exerted by honey and its major components such as chrysin and other flavonoids (Gheldof & Engeseth, 2002; Mabrouk et al., 2002; Swellam et al., 2003; Tonks et al., 2001; Woo, Jeong, Park, & Kwon, 2004). Honeys from various floral sources have been shown to be rich in phenolic compounds, which are substances known to mediate oestrogen effects via modulation of the oestrogen receptor activity, being oestrogen agonists or antagonists in a cell and tissue type specific manner (Gomez-Caravaca, Gomez-Romero, Arraez-Roman, Segura-Carretero, & Fernandez-Gutierrez, 2006; Merken & Beecher, 2000; Moutsatsou, 2007; Schramm et al., 2003; Yao et al., 2004). Despite the high phenolic content of honeys, there is scant information regarding the potential of honey extracts to induce oestrogenic effects and to activate oestrogen receptor mediated activity. Moreover, the effects of honeys on hormone dependent cancers such as breast, endometrial and prostate cancer remain largely unknown.

The rich Greek flora biodiversity, with a high percentage of endemic plants, includes a wide variety of honeys emanating from thyme, pine, fir and other conifers. Therefore, it was interesting to examine the oestrogenic/antioestrogenic activity of Greek honeys (thyme, pine and fir tree) from various regions in Greece by using *in vitro* assays widely accepted to assess oestrogenic responses such as the ability: (1) to induce luciferase activity in MCF-7 cells co-transfected with an ERE-driven Luciferase reporter gene and (2) to modulate the cell viability/proliferation of Ishikawa cells, MCF-7 cells and PC-3 cells by using the MTT-cell viability assay. Given that the chemical composition of Greek honeys is largely unknown, and because existing data are limited to comparison of the volatile composition in thyme honeys from several origins in Greece (Alissandrakis, Tarantilis, Harizanis, & Polissiou, 2007), we further determined in these honeys the main profiles of chemical

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composition (total phenolics, phenolic acids, sugars and fatty acids).

## 2. Materials and methods

### 2.1. Chemicals

MCF-7 and PC-3 cell lines were purchased from ATCC Cell Bank, Ishikawa cell line was purchased from ECACC Cell Bank. All cell-culture materials, such as Dulbecco's minimal essential medium (DMEM), foetal bovine serum (FBS), trypsin – EDTA solution, penicillin, streptomycin and L-glutamine were obtained from Gibco BRL (Thessalonica, Greece). 17β-estradiol ($E_2$) (E4389), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; M5655), gallic acid, phenolic acids (syringic, vanillic, sinapic, benzoic, chlorogenic, gallic, p-coumaric, cinnamic, caffeic, ferulic, p-hydroxybenzoic and protocatechuic), flavonols (myricetin and quercetin) and 5-(hydroxymethyl)-2-furaldehyde (hydroxymethylfurfural; HMF), Folin–Ciocalteús phenol reagent and other chemical materials were purchased from Sigma Aldrich (St Louis, MO, USA). Sugar standards (glucose, fructose and sucrose) were purchased from Merck, Baker. Methylated fatty acid (FAME) standard GLC-461 was supplied from Nu-chek Prep Inc. (MN, US). ICI182780 was obtained from Tocris. B-galactosidase (pCMVβ) reporter plasmid was obtained from Clontech (Athens, Greece) and polyfect transfection reagent was obtained from Qiagen (Athens, Greece). The Luciferase assay system and the β-galactosidase enzyme assay system were obtained from Promega (Athens, Greece).

### 2.2. Preparation of honey extracts

The honey samples were collected in the island of Crete (Iraklion, South Greece), Karpenisi (North–West Greece) and in the island of Euboea (Central East Greece). The samples were subjected to pollen analysis, resulting in concentration of pollen from *Thymus* sp., of *Abies cephallonica* and of Pinaceae family (*Pinus* sp.), respectively. Voucher samples (pine honey Euboea, fir honey Karpenisi and thyme honey Crete) have been deposited at the Laboratory of Pharmacognosy–Chemistry of Natural Products, School of Pharmacy, University of Athens, Greece.

Each honey sample (1 kg) was diluted with water (1 l) and the solution was extracted 3× with ethyl acetate (1 l). The ethyl acetate phase was collected and evaporated to dryness affording 25.2 g of pine honey, 22.4 g of fir honey and 30.3 g of thyme honey. Dried ethyl acetate honey extracts (ca. 100 mg) were diluted in 2 ml methanol/water (1:1) prior to analyses.

### 2.3. Quantitative analysis of total phenolics

Total phenolic content was determined by Folin–Ciocalteau method (Wrolstad et al., 2005), where gallic acid was used as a calibration standard in spectrophotometric measurement.

### 2.4. Chromatographic determination of the phenolic profile of honeys and quantitative analysis of hydroxymethylfurfural (HMF)

Samples were diluted fivefold with water, filtered (0.45 μm) and analysed by HPLC (high-performance liquid chromatography) (Agilent 1100 Series HPLC–MSD, Agilent Technology) connected to a diode array detector (DAD) and mass selective detector (MSD, API–ESI). Analyses were performed on a HyperClone ODS (C18) column (2.0 mm × 200 mm, 5 μm, Phenomenex) using a gradient run (0.2 ml/min) with methanol (A) and 0.3% formic acid (B). The linear gradients used were: 90–75% B from 0 to 9.5 min, 75–67.5% B from 9.5 to 22 min and 67.5–40% B from 22 to 40 min. The run was stopped at 70 min and post time was 20 min. Phenolic acids were determined at 260 nm, flavonols at 340 nm and 5-(hydroxymethyl)-2-furaldehyde (HMF) at 280 nm. Authentic standards of phenolic acids, HMF and flavonols were used for identification and quantitation.

### 2.5. Chromatographic determination of the profile of volatile compounds and fatty acids

Fatty acids and volatile compounds were analysed by GC–MSD (gas chromatography–mass selective detector) (HP6890, HP5973, Agilent Technologies) with EI ionisation on a HP–FFAP (polyethylene glycol TPA) column (25 m, 0.20 mm, 0.33 μm). Fatty acids were methylated prior to GC–MSD analysis using a method reported by Prevot and Gantois (1975), with some modifications. Volatile compounds were injected (1 μl) in spilt mode (ratio 40:1) at 250 °C and helium (purity 6.0, AGA, Finland) was used as a carrier gas. The GC oven temperature was held at 75 °C for 6 min, increased at 1.5 °C/min to 200 °C and held for 10 min. Post run was performed at 220 °C for 5 min. Compounds were identified using Wiley 275 library database.

### 2.6. Quantitative analysis of sugars

Samples were diluted with water (1:10 v/v) and analysed by HPLC (HPLC 1100, Agilent Technologies) using an IC Sep ICE-ORH-801 column (6.5 mm × 300 mm, Transgenomic). An isocratic run was performed with 0.0025 N sulphuric acid. Sugars were detected with an RI-detector. Quantitative results for sugars were calculated with authentic standards.

### 2.7. Plasmids and transfections

MCF-7 cells were grown in DMEM supplemented with 10% FBS. Stock cultures were subcultured every 4–5 days using a trypsin (0.25%) – EDTA (0.02%) solution. Before each transfection experiment, cells were maintained for 2 days in DMEM, phenol red (PR) free, containing 10% dextran-coated charcoal stripped serum (FBS DCC treated). For each transfection experiment $2 \times 10^5$ cells were plated per well in six-well dishes in DMEM PR (−) with 10% FBS (DCC treated). After 24 h, MCF-7 cells were transfected with 0.75 μg β-galactosidase (pCMVβ) reporter plasmid and 0.75 μg ERE (3X ERE-TATA-Luc) expression vectors by using the polyfect transfection reagent according to manufacturer's guidelines. After 24 h, cells were washed once with phosphate buffer saline (PBS) and 2 ml of DMEM PR (−) 10% FBS (DCC treated) was added, containing a final concentration of $E_2$ ($10^{-9}$ M) or ICI182780 ($10^{-8}$ M) or extracts (0.2–100 μg/ml). Co-incubation of $E_2$ ($10^{-9}$ M) with extracts (0.2–50 μg/ml) or ICI182780 ($10^{-8}$ M) was also done. Cells were harvested 24 h later and the cell extracts were assayed for luciferase activity, using the Promega luciferase assay system and for β-galactosidase activity, using the β-galactosidase enzyme assay system. B-galactosidase expression plasmid was co-transfected in the assay system as an internal standard to normalise the luciferase activity.

### 2.8. MTT-cell viability assay

Ishikawa cells, MCF-7 cells and PC-3 cells were maintained in DMEM supplemented with 10% FBS, 50 units/ml penicillin and 50 μg/ml streptomycin in T-75 cm$^2$ flasks at 37°C, 85% humidity, and 5% $CO_2$ atmosphere. Subcultures were carried out every 3–4 days using a 0.25% trypsin −0.02% EDTA solution.

The cell viability was estimated by a modification of the MTT assay (Denizot & Lang, 1986), which determines the metabolically active mitochondria of cells. Cells were plated in their growth med-

ium at a density of 10,000 cells/well in 96 flat-bottomed well plates. After 24 h plating, test extracts were added at final concentrations ranging from 0.2 to 125 μg/ml in DMEM PR (−). After 48 h incubation, the medium was replaced with MTT dissolved at a final concentration of 1 mg/ml in serum-free, PR free medium, for further 4 h incubation. Then the MTT-formazan was solubilised in isopropanol and the optical density was measured at a wavelength of 550 nm and a reference wavelength of 690 nm. The MTT-cell viability assay depends on the reduction by the mitochondria of living cells of MTT to form a blue formazan product. Thus, results depend both on the mitochondria activity per cell and on the number of cells present, therefore it is a very useful assay for cell proliferation and survival.

### 2.9. Statistical analysis

The degree of agonism/antagonism for each concentration of test extracts was calculated and expressed as percentage relative to the response of the control. All values are expressed as the mean ± standard deviation of four (transfection assay) or three (MTT assay) measurements. Statistical analysis was performed using a *t*-test two-tailed distribution, assuming two-sample unequal variance. A $P < 0.05$ value was considered statistically significant ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

## 3. Results and discussion

The present study was undertaken with the goal to determine the oestrogenic/antioestrogenic properties of Greek honey extracts derived from thyme, pine and fir honey, as well as their effects on cell viability/proliferation of breast, endometrial and prostate cancer cells. We also investigated whether the chemical composition (total phenolics, phenolic acids) of Greek honeys could account for the observed biological effects.

### 3.1. Composition of honey extracts

Table 1A provides the composition of Greek honey extracts expressed per 100 g extract. Considering the amount of extract derived from 1 kg honey (30.3 g, 25.2 g, 22.4 g for thyme, pine and fir honey, respectively), as described in Section 2, and taking into account the composition of honey extracts per 100 g extract, the composition of Greek honeys were also expressed per 1 kg honey (Table 1B).

**Table 1**
Composition of the Greek honey extracts (expressed per 100 g extract) and composition of Greek honeys (expressed per kg honey)

| | Thyme (%SD) ($n = 5$) | Fir (%SD) ($n = 5$) | Pine (%SD) ($n = 5$) |
|---|---|---|---|
| *(A) Phenolic compounds (mg/100g)* | | | |
| Total phenolics | 990(8) | 510(10) | 1160(17) |
| *Sugars (g/100 g)* | | | |
| Sucrose (g/100 g) | 3 (22) | 6 (23) | 3 (38) |
| Glucose (g/100 g) | 17 (15) | 21 (20) | 10 (11) |
| Fructose (g/100 g) | 38 (25) | 42 (15) | 41 (18) |
| Hydroxymethylfurfural (mg/100 g) | 670 (81) | 134 (114) | 49 (103) |
| *(B) Phenolic compounds (mg/kg honey)* | | | |
| Total phenolics | 300 | 114 | 292 |
| *Sugars (g/kg honey)* | | | |
| Sucrose (g/kg honey) | 0.9 | 1.34 | 0.75 |
| Glucose (g/kg honey) | 5.15 | 4.7 | 2.52 |
| Fructose (g/kg honey) | 11.51 | 9.4 | 10.33 |
| Ratio fructose/glucose | 2.2 | 2 | 4 |
| Hydroxymethylfurfural (mg/kg honey) | 203 | 30 | 12.34 |

SD, standard deviation.

As shown in Table 1A, Greek honey extracts are rich in total phenolic compounds (510–1160 mg/100 g extract), including phenolic acids. Thyme and pine honey had a higher total phenolic content (ca. 1000 mg/100 g extract) than fir honey (510 mg/100 g extract). The total phenolic content was, for thyme honey 300 mg/kg honey, for pine honey 292 mg/kg honey and for fir honey 114 mg/kg honey. Comparing our data with the total phenolic content of honeys derived from other flora sources (range 46–400 mg/kg honey, mean 270 mg/kg honey) (Gheldof & Engeseth, 2002), we conclude that the Greek honeys examined here are rich in phenolic content. Fir and pine honey contained protochatechuic acid, whereas p-hydroxybenzoic acid and vanillic acid were found in all honey extracts (Fig. 1). The levels of p-hydroxybenzoic acid and vanillic acid were higher in thyme honey than in other honey extracts (semi-quantitative results). Similar phenolic acids have been reported to be present in honeys of other botanical origin (Gomez-Caravaca et al., 2006; Jerkovic, Mastelic, & Marijanovic, 2006). Honey extracts contained also a number of unidentified compounds (UI-U17 in Fig. 1), which were probably phenolic acids or their derivatives (based on DAD spectrums). Quercetin and myricetin were not present. All honey extracts contained sugars; sucrose ranged from 0.75 to 1.34 g/kg honey, glucose ranged from 2.5 to 5.1 g/kg honey and fructose ranged from 9.4 to 11.5 g/kg honey (Table 1B), values similar to those reported for honeys derived from other floral sources. More importantly the fructose/glucose ratio (mean 2.5) fell in the same range as that of other honeys (Oddo et al., 2008). Hydroxymethylfurfural (HMF) was present in all Greek honeys at concentrations similar to those found in commercial honeys, nectar honeys and honeys obtained directly from beekeepers (Sanz, del Castillo, Corzo, & Olano, 2003). We estimated that the HMF contents of pine honey and fir honey were 12.34 mg/kg honey and 30 mg/kg honey, respectively (Table 1B), lower than allowable limit of 40 mg/kg honey according to composition criteria for honey (Official Journal of the European Communities, 2002; Sanz et al., 2003). However, HMF was present in abundance in thyme honey (203 mg/kg honey), probably related to its long period of storage in the laboratory. The GC–MSD profiles of all honey extracts were similar (Fig. 2). The major compounds in GC–MSD analyses were 1,3-dihydroxypropanone and HMF in all extracts. Fir and thyme honey contained more early eluting volatile compounds (peaks 1–6 in Fig. 2) than pine extract, whereas pine honey contained more late eluting volatile compounds (peaks 8, 9, 11, 14 in Fig. 2) than other extracts. The volatile compounds detected in Greek honey extracts were in agreement with other reports (Alissandrakis et al., 2007). Fatty acids were not present in honey extracts.

### 3.2. Transfection assay

For the assessment of oestrogenic potency, transiently transfected MCF-7 cells were used with an ERE-driven luciferase reporter gene assay, which includes the most important steps in oestrogen signalling and it is a well-recognised *in vitro* assay for the evaluation of the oestrogenic effect of extracts (Mueller, 2002). Oestrogen receptors bind to estrogens, dimerize and translocate into the nuclei where they bind directly onto DNA at specific base sequences, the oestrogen-response elements (EREs), thus regulating the transcription of oestrogen responsive genes and the translation of specific proteins, which elicit the oestrogenic effect in the target cells. This signalling cascade induced by estrogens (endogenous or plant-derived) may be modulated at any stage and defines the oestrogenic/antioestrogenic potency of a tested compound or extract. The association of the liganded ER dimer to the so-called consensus ERE is a key step in the oestrogen-induced biological effects, therefore the transfection assay was used to evaluate the oestrogenic activity of honey extracts. $E_2$ and ICI 182780 were used as a positive and negative control, respectively.

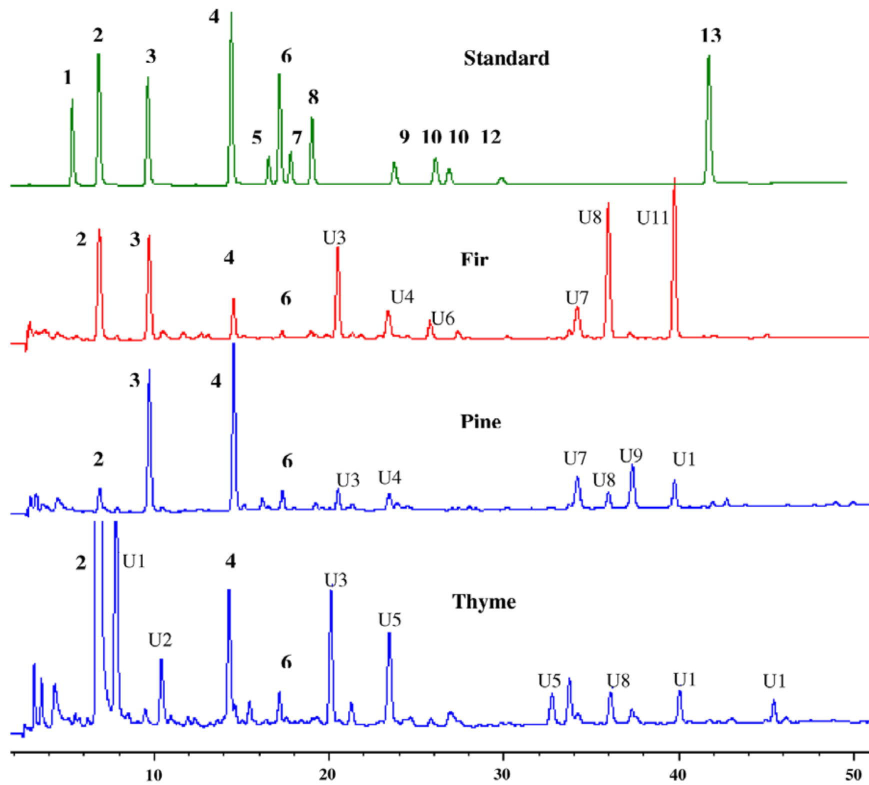


**Fig. 1.** HPLC–DAD chromatograms (260 nm) of honey samples and a standard mixture of phenolic acids and hydroxymethylfurfural (HMF). Peak identification: (1) gallic acid; (2) HMF; (3) protocatechuic acid; (4) p-hydroxybenzoic acid; (5) chlorogenic acid; (6) vanillic acid; (7) caffeid acid; (8) syringic acid; (9) coumaric acid; (10) ferrulic acid; (11) sinapic acid; (12) benzoic acid; (13) cinnamic acid; U1-U12) unidentified peaks.

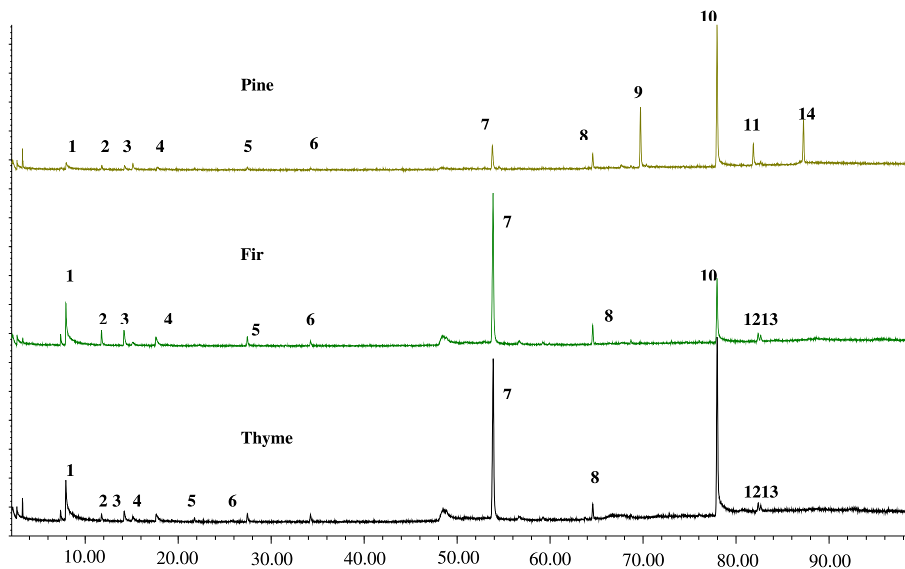


**Fig. 2.** GC–MSD chromatograms of honey samples. Peak identification (Wiley 275 database): (1) hydroxyacetaldehyde; (2) unknown; (3) acetic acid; (4) formic acid; (5) 2-furanmethanol; (6) 2-hydroxycyclopent-2-en-1-one; (7) 1,3-dihydoxypropanone; (8) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; (9) monomethylsuccinate; (10) HMF; (11–13) unknown; (14) 1,2-benzenedicarboxylic acid dibutyl ester.

In order to decide the proper dosages of honey extracts to be tested in our *in vitro* system, we took into account the following: (a) the weight of a teaspoon of honey is approximately 30 g; (b) our data on honey extract preparation indicate that 1 kg of each honey sample result in 22–30 g extract and (c) that each extract contains phenolic compounds ranging from 500–1100 mg/100 g dry extract. Considering the above, the human body weight, the blood volume as well as the absorption and bioavailability of dietary phenolics (Ross & Kasum, 2002), the honey extracts were tested at concentrations (0.2–100 μg/ml) which are physiologically achievable and correspond to concentrations of phenolic compounds which are within the range of expected physiological plasma levels of dietary phenolics (Ross & Kasum, 2002; Wiseman, 1999). Furthermore, a concentration range from 0.2 to 100 μg/ml

is within the reported range of other plant extracts which have also been shown to activate transcription of ERE-dependent genes, via both ER subtypes (Ju et al., 2000).

MCF-7 cells, transiently transfected with an ERE-driven luciferase reporter plasmid and a β-galactosidase reporter plasmid, were incubated with the extracts of thyme honey, pine honey and fir honey at concentration 0.2–100 μg/ml. Co-incubation of extracts (0.2–50 μg/ml) in presence of $E_2$ ($10^{-9}$ M) was also done. The results of the luciferase activity are expressed as a percentage (%) of the vehicle control (cells incubated in absence of extracts or compounds) and presented normalised to β-galactosidase activity in Fig. 3a–c. Thyme honey at 20–100 μg/ml, when examined alone, demonstrated a significant oestrogenic effect ($P < 0.01$), whilst at lower concentration (2 μg/ml) it showed a significant antioestrogenic effect ($P < 0.001$). Its co-incubation with $E_2$ ($10^{-9}$ M) inhibited significantly the $E_2$-stimulated luciferase gene induction ($P < 0.001$), implicating thus its $E_2$-antagonistic action (Fig. 3a). Pine honey extract, when examined alone, exhibited a significant oestrogenic effect at concentration of 20 μg/ml ($P < 0.01$) and at 50-100 μg/ml ($P < 0.001$). However, it showed a significant antioestrogenic effect at lower concentrations (2-5 μg/ml) when examined alone in the system ($P < 0.05$) and when co-incubated with $E_2$ ($10^{-9}$ M) ($P < 0.05$), suggesting thus its antioestrogenic activity (Fig. 3b). Fir honey extract at 20–100 μg/ml, when examined alone, exhibited a significant oestrogenic effect ($P < 0.001$), whereas at concentration 0.2–5 μg/ml it showed significant antioestrogenic effect ($P < 0.05$ and $P < 0.01$, respectively). Its co-incubation (0.2–50 μg/ml) with $E_2$ ($10^{-9}$ M) did not inhibit the $E_2$-stimulated luciferase gene induction ($P < 0.05$) (Fig. 3c).

Co-incubation of $E_2$ ($10^{-9}$ M) with ICI 182780 ($10^{-8}$ M) reduced the stimulatory effect of $E_2$ in a statistically significant way as expected ($P < 0.001$) (Fig. 3a–c).

The present findings suggest that the honey extracts exhibit a biphasic activity, being significant oestrogen agonists at high concentrations (20–100 μg/ml) but oestrogen antagonists at low concentrations (0.2–5 μg/ml) when examined alone in the system. The honey extracts contain a mixture of polyphenolic compounds and multiple effects (synergistic or antagonistic) may occur at different dosages, giving their bifunctional activities. In line with our findings, cruciferous vegetable extracts or commercial herbal formulas act bifunctionally i.e. as antioestrogens at low concentrations and as oestrogen agonists at high concentrations (Ju et al., 2000; Moutsatsou, 2007). However, pine and thyme honey exhibit antagonistic activity when presented with estradiol, whereas fir honey shows a super agonist effect on the $E_2$ activity. In line with these findings, previous reports have shown that individual flavonols such as quercetin and kaempherol, when alone, transactivate ER activity whereas in presence of $E_2$ (0.5 nM) act as oestrogen antagonists (Harris, Besselink, Henning, Go, & Heber, 2005; Moutsatsou, 2007). A "super agonist" effect on the activity of $E_2$ has also been seen with various phenolic compounds (Harris et al., 2005). Taken together, the high phenolic content may explain, at least in part, the oestrogenic/antioestrogenic activity in MCF-7 cells demonstrated by the Greek honey extracts. More importantly, the bifunctional activities of honey extracts point out that they may play an important role in oestrogen-dependent diseases i.e. they may be useful to prevent symptoms associated with oestrogen deficiency during menopause or may be beneficial in breast cancer risk by antagonizing the oestrogen action.

### 3.3. Cell viability study

A main characteristic of most cancers including breast, endometrial and prostate cancer is an uncontrolled cellular growth whilst substances that monitor cell proliferation may be useful in cancer prevention. Estrogens are known stimulants of breast cancer whereas the viability/proliferation of MCF-7 cells is a well-established biological test for screening extracts that may function as oestrogen agonists/antagonists. The MTT assay assesses indirectly the effect of a substance or extract on the cellular survival and proliferation.

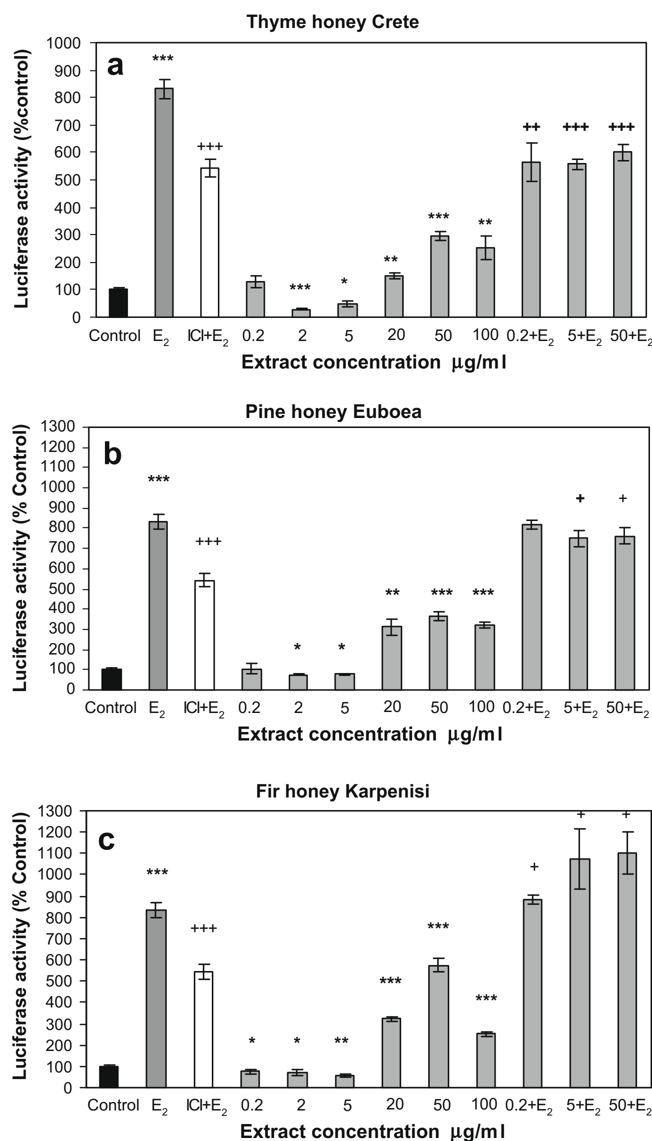


**Fig. 3.** The effect of thyme honey extract (a) or pine honey extract (b) or fir honey extract (c) on luciferase activity in MCF-7 cells transfected with β-gal and EREs. MCF-7 cells were treated with $E_2$ ($10^{-9}$ M) and test extracts (0.2–100 μg/ml). Co-incubation of $E_2$ ($10^{-9}$ M) with extract (0.2–50 μg/ml) or ICI 182780 ($10^{-8}$ M) was also done. Results of luciferase activity are expressed as percentage of control (cells incubated in absence of extracts) and presented normalised to β-galactosidase activity. Columns and bars represent mean ± standard deviation of the results of four measurements. *Significant difference ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) vs. vehicle control; +Significant difference ($^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$) vs. $E_2$.

The dose response curve concerning the effect of honey extracts on the viability of Ishikawa, MCF-7 and PC-3 cells is shown in Fig. 4. Thyme honey reduced significantly the Ishikawa and PC-3 cell viability implicating that it may be useful for the prevention of prostate and endometrial cancer. Pine and fir honey extracts at a concentration range 0.2–125 μg/ml did not show any significant effect on Ishikawa and PC-3 cell viability. MCF-7 cells showed no response to pine and thyme honey whereas, fir honey stimulated the viability of MCF-7 cells.

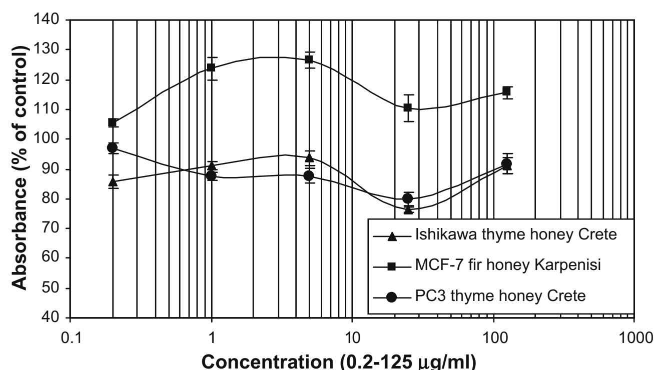


**Fig. 4.** Effect of thyme honey extract on cell viability of Ishikawa and PC-3 cells and fir honey extract on MCF-7 cells. Ishikawa, MCF-7 and PC-3 cells were incubated with the indicated concentrations of extracts (0.2–125 μg/ml) for 48 h. Cell viability was determined by the MTT assay. Each point of the dose response curve is the average of three measurements and expressed as percentage of control (cells incubated in the absence of extracts).

Given that polyphenols and phenolic acids (vanillic acid and protocatechuic acid and p-hydroxybenzoic acid) are known to inhibit cancer-related pathways and processes (Kris-Etherton et al., 2002; Yech, Huang, & Yen, 2005; Yech & Yen, 2005), including prostate cancer (Chan, Gann, & Giovannucci, 2005; Von Low, Perabo, Siener, & Muller, 2007) and endometrial cancer (Burton & Wells, 2002), we looked for features, e.g. the chemical composition that could account for the differences between the honey extracts in viability/proliferation effects. In this respect, the high phenolic content in thyme honey compared to the low phenolic in fir honey, may, in part explain, the growth inhibitory effects of thyme honey vs. the growth stimulation effects of fir honey in cancer cells. In view of recent data that hydroxymethylfurfural might have antitumour potential, the abundance of hydroxymethylfurfural in thyme honey implicates its possible role in the biological activities observed (Michail et al., 2007). Our data extend the results of previous investigators demonstrating that plant extracts and herbal mixtures, which are rich in total phenolics, inhibit the cellular growth of human prostate cancer cells (Adhami & Mukhtar, 2006).

## 4. Conclusions

Some varieties of Greek honeys, especially thyme honey, are very rich in compounds known to possess anticancer properties, such as polyphenols, and phenolic acids. Greek honey extracts exhibit significant biological effects in human cancer cells, such as antioestrogenic activity in MCF-7 cells and inhibition of cell viability on prostate cancer and endometrial cancer cells. We suggest that honey, especially the thyme honey, may be used as an alternative to sugar, whilst its incorporation in the human diet or as a food ingredient may prevent cancer-related processes, promoting thus the health of consumers.

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