Olive Oils from Corfu Island (Greece): Content and Antimicrobial Activity of Health-Protective Compounds and Antioxidant Activity of the Oils.

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Introduction

Health benefits from extra-virgin olive oil have been documented through several epidemiological studies. Many beneficial pharmacological activities are attributed to olive oil's polyphenols. Oleocanthal (Compund A) and oleacein (Compound B), have recently gained attention as possessing anti-inflammatory and anti-rheumatic activities and being neuroprotective and chemotherapeutic. Regulation EU432/2012 allows of a health clain "Olive oils polyphenols contribute to protection of blood lipids from oxidative stress" for extra-virgin olive oils containing at least 5 mg of hydrotyrosol and derivatives /20 g of olive oil.

Aim

The aim of this work was to determine the content in six phenolic compounds with health-protecting potential, probe the antioxidant activity and its correlation with the phenolics and examine the antimicrobial activity of oil and of each specific phenolic.

Methods

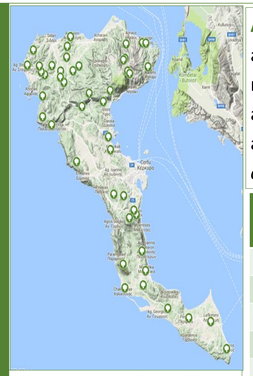
Olives were collected late October (semi-mature) for extra-virgin olive oil. 3 kg olive samples. Only healthy fruits were crushed, pitted, blended, homogenized (45 min) and centrifuged (4000 rpm, 5 min).
Temperature was kept <27°C during the process. Samples were stored at -20oC.

Determination of six phenolic compounds namely, oleocanthal (A), oleacein (B), ligstroside aglycone (C), oleokoronal (D), oleuropein aglycone (E) and oleomissional (F), was done via 1D - 1H-NMR spectroscopy. Antioxidant activity was determined spectrophotometrically by measuring the decrease of the DPPH absorbance at 517 nm, after addition of 6 µl of olive oil sample in 3 ml of a 100 µM DPPH solution in ethyl acetate.
The phenolic compounds concentration is expressed in mg/Kg and the antioxidant activity in Trolox equivalents (mmol Trolox/l). Experiments were performed in triplicate.
The antimicrobial activity of an olive oil sample as well as of compounds A, B, C, E, and F was tested on Listeria monocytogenes ATCC 15313, 7644 and 1911, Bacillus cereus ATCC 14579 and 10876 and Staphylococcus aureus ATCC 25923 via the disc assay.

Disc Assay: Bacterial cells were mixed with soft agar and placed onto Mueller Hinton agar plates. Sterile discs were placed and 5.0, 2.5, 1.3, 0.063, 0.032 and 0.016 µg of appropriate substance diluted in ethanol was pipetted on individual discs.
The lowest concentration that showed a zone of inhibition was used to measure the antimicrobial activity of the tested substances. Modification of the standard disc assay was needed to avoid interference of the hydrophobicity of the substances with the results.

The inhibitory activity of pure ethanol was examined as well and erythromycin was used as a positive reference standard. All tests were done in triplicates.
The data analysis and graphical displays were done via the program OriginPro 7.5 (Origin Lab) and the statistical analysis of the results via SPSS v.20

Results and Conclusion



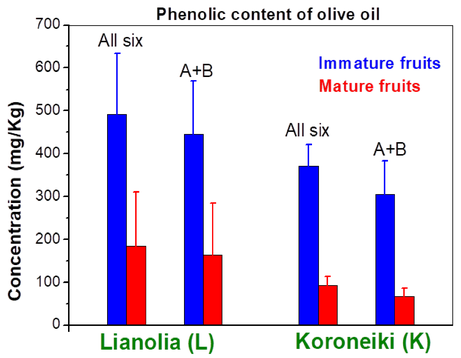
Care was taken during olive harvesting to obtain samples representing the whole island Figure above shows the geographical distribution of the olive samples collected.

Antimicrobial activity is possessed in all compounds, except ligstroside aglycone. Oleocanthal was inhibitory for all strains at 0.063 µg. Oleacein was required at 0.063 µg to cause inhibition to the two *Bacillus cereus* strains and at 1.3 µg to produce the same effect to the strains of *Listeria monocytogenes* and *Staphylococcus aureus*. Oleuropein aglycone inhibited only *Bacillus cereus* at 1.3 µg.

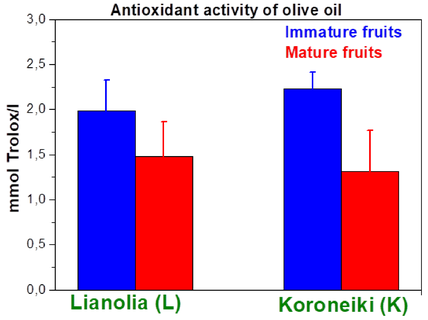
Compound (µg/disc)	Microorganism (ATCC Strain)					
	15313	19111	7644	10876	14579	25932
Oleocanthal	0.063	0.063	0.063	0.063	0.063	0.063
Oleacein	1.3	1.3	1.3	0.063	0.063	1.3
oleuropein aglycone	>5.0	>5.0	>5.0	1.3	1.3	>5.0
ligstroside aglycone	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0
Olive Oil	0.032	0.032	0.032	0.032	0.032	0.032
Erythromycin	0.063	0.032	0.032	0.063	0.063	0.063

No inhibition was observed for ethanol

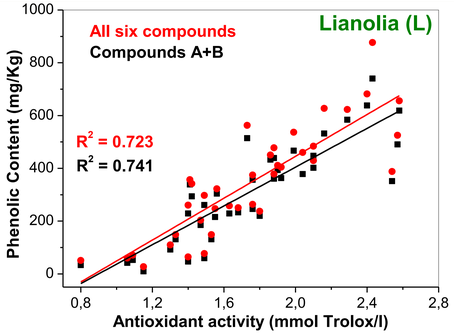
Determination of phenolic content and antioxidant activity: on 49 olive oil samples from cultivars "Lianolia" ("L", 38 samples - 20 derived from immature and 18 from mature fruits) and "Koroneiki" ("K", 11 samples - 3 derived from immature and 8 from mature fruits respectively).



The mean **phenolic content** from immature fruits was higher relative to mature fruits by a factor of ca. 2.7 and 4.3 for the cultivars "L" and "K". For "L" and "K" (same maturity) were the same (95% of statistical significance). Caution: small number of samples for cultivar K.



The **mean antioxidant activity** from immature fruits was higher than mature fruits by ca. 1.3 and 1.7 for the cultivars "L" and "K". For "L" and "K" (same maturity) was similar (95% of statistical significance). Caution: small number of samples for "K". Dependence of olive oil antioxidant activity on the cultivar type and fruit maturity level was the same with the phenolic content.



Correlation between phenolic content and the olive oil antioxidant activity. For "L" significant linear dependence between antioxidant activity, combined concentration of phenolic compounds A and B (R^2=0.741) and summative concentration of all measured phenolic compounds A to F (R^2=0.723). Highest correlation for compound B -oleacein (R^2=0.748). For "K" similar but less pronounced linear correlation between antioxidant activity and phenolics concentration; R^2=0.555 (combined concentration of compounds A+B) and 0.527 (summative concentration of all six measured phenolics).

Conclusions

Olive oil from Corfu island and specific isolated phenolic compounds were shown to possess antimicrobial activity. The olive oils of both cultivars ("Lianolia" and "Koroneiki") extracted from immature fruits exhibited significantly higher antioxidant activity as well as phenolic concentration relative to those extracted from immature fruits. The antioxidant activities of both cultivars were shown to be linearly correlated with their phenolic content, with the effect being more pronounced for the cultivar "Lianolia".

Acknowledgment

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