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Short Communication

Cytostatic activity of some compounds from the unsaponifiable fraction obtained from virgin olive oil

M.T. Saenz [a,*], M.D. Garcia [a], M.C. Ahumada [a], V. Ruiz [b]

[a] *Laboratorio de Farmacognosia, Facultad de Farmacia, Universidad de Sevilla, 41012 Seville, Spain*

[b] *Instituto de la Grasa (CSIC), Seville, Spain*

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Abstract

Oleuropein, tyrosol, squalene and the fraction of sterols and triterpenoid dialcohols from the unsaponifiable fraction obtained from virgin olive oil have been tested for its possible cytostatic activity against McCoy cells, using 6-mercaptopurine as a positive control. The samples of sterols and triterpenic dialcohols showed a strong activity.

Keywords: Olive oil; Cytostatic activity; Oleuropein; Tyrosol; Sterols

1. Introduction

Epidemiological and experimental studies suggest that some nutrients, including phenolic substances, play an important role in the prevention of various diseases. However, the widespread distribution of phenolic substances in natural vegetable oils as a rich source is dramatically decreased after processing to make them edible [1,2].

Physiological benefits are probably determined by the large amount of minor components in the unsaponifiable fraction of virgin olive oil, which include carotenes, phenols, sterols and triterpenoids [3,4].

The main phenolic compound in the olive is oleuropein and the effects on the heart lipids of the rat have been studied [5,6].

The diversity of biological activity of sterols and triterpenoids has also been studied and include the development and control of the reproductive tract in man, the molting of insects and the induction of sexual reproduction in aquatic fungi. In addition, steroids contribute to a wide range of therapeutic applications such as cardiotonics, oral contraceptive agents, antiinflammatory agents and anabolic agents [7,8].

In this study we have examined 'in vitro' the effects produced by these minor components (oleuropein, tyrosol, squalene, sterols and the triterpenic fraction) on cultures of McCoy cells.

* Corresponding author. E-mail:saenz@farfar.us.es

These cells were reported to have originated from the synovial fluid in the knee joint of a patient suffering from degenerative arthritis.

2. Experimental

2.1. Plant material

Olea europaea L. was collected in Seville (Spain). The fruit of fresh olives was extracted twice with water and refluxed gently for 10 min. The separation and purification have been described in a previous report [9].

The samples (oleuropein, tyrosol, squalene, sterols and the triterpenic fraction) were provided by the Instituto de la Grasa (CSIC), Seville, Spain.

2.2. Cytostatic test procedure

The cytostatic activity was determined by measuring the inhibition of the development of a single-layer culture of McCoy cells (ATCC CRL 1696) cultivated in Eagle's essential minimum medium (MEM), according to the method described by Geran et al. [10]. Cells were grown in MEM supplemented with 5% of bovine fetal serum and a 2% solution of penicillin and streptomycin (5000 IU/ml, 5000 μm/ml) at pH 7.2 and 36°C. After distribution in the nutritional

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medium and when a continuous monolayer culture had been obtained, the samples were sterilized through a 0.22 μm Millipore filter and then inoculated.

Oleuropein and tyrosol were dissolved in a 1% hydroalcoholic solution as vehicle, and squalene, sterols and the triterpenic fraction in a 1% Tween 80 solution. Initially, the samples were diluted to give a concentration of 6 μg/ml (ID_{50} values recommended by the National Cancer Institute of USA for pure compounds). Subsequently, as a consequence of the results, the assayed dose was between 1–10 μg/ml. A solution of 6-mercaptopurine as a positive control [11] and a 1% hydroalcoholic solution as a blank control were used under identical conditions.

72 h after inoculation of the samples, incubated at 36°C, the cellular protein concentration was determined to evaluate the inhibitory effect on growth. The colorimeter method of Bradford [12] was followed, using a calibration gauge with different concentrations of a standard solution of human albumin. Each assay was carried out in triplicate and the average of the readings was recorded.

3. Results and discussion

In this paper, we studied the cytostatic effect of different doses of these minor components of virgin olive oil.

As can be observed, at the dose of 6 μg/ml (Table 1), the samples of oleuropein, sterols and the triterpenic fraction exhibited a higher degree of growth inhibition than 6-mercaptopurine solution used as a positive control. These samples showed almost similar activity (83.63 and 89.40%, respectively). Tyrosol exhibited minor inhibition (34.61%) and squalene was inactive against the cell line employed.

The ID_{50} for oleuropein (4.40 ± 0.17), sterols and the triterpenic fraction (0.11 ± 0.01) are lower than those recommended by the Protocols of the National Cancer Institute of USA [10] (Table 2).

The high cytostatic activity observed in oleuropein is probably due to the phenolic structure [13].

The inhibition of cellular growth showed by sterols and triterpenics from different botanical families has been studied by various authors in several cell cultures [14–16].

For this reason, more assays are being performed with the sterols and triterpenics fraction in order to confirm their activity against experimental tumors in vivo.

Consequently, this fraction obtained from the unsaponifiable fraction of virgin olive oil could be considered as a potentially cytostatic agent in inflammatory and tumoral diseases.

Table 1
Cellular inhibition (%) with the dose of 6 μg/ml

Compound tested	Cellular inhibition (%)
Sterols and triterpenic fraction	89.41
Oleuropein	83.63
Tyrosol	34.61
Squalene	–

Table 2
Cytostatic activity on McCoy cells

Compound tested	ID_{50} (μg/ml)
Sterols and triterpenic fraction	0.11 ± 0.01
Oleuropein	4.40 ± 0.17
Tyrosol	10.01 ± 0.46
Squalene	> 200
6-Mercaptopurine	0.43 ± 0.03

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