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Pubblichiamo una selezione di alcuni degli interventi presentati in occasione dell'evento organizzato da S.I.Fit.

Per ciascun intervento sono indicati l'autore, o gli autori, e la relativa qualifica.

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IL TÈ VERDE NELLA CHEMOPREVENZIONE ONCOLOGICA

The constant increase in the incidence of prostate cancer (CaP) in Western Countries has brought the disease to rank now second as leading causes of cancer-related death among men. Therefore it represents a major health and social problem. CaP is a slow progression disease and very often diagnosis is made late in elderly men. For this reason, CaP is an ideal target for chemoprevention strategies. At present, chemoprevention may be the best approach to fight CaP. A growing body of evidence has suggested that biologically active compounds from green tea (catechins: the most common are EGCG, EGC, ECG and EC) might possess anti-tumour activity. Recently we showed that a Green Tea Catechins extract (GTCs) was very effective at inhibiting cancer growth in vitro and in a transgenic animal model (A. Caporali, et al., 2004). In our hands, GTCs triggered death in

cancer cells probably through induction of the nuclear form of Clusterin. Although results from the laboratory were promising, no definitive clinical data demonstrating the efficacy of GTCs at inhibiting CaP progression in humans were still available. Thus, in 2006 we performed a clinical trial in a cohort of 60 human volunteers bearing HG-PIN, the main pre-malignant lesion of CaP, demonstrating that about 90% inhibition of CaP development was achievable by oral administration of GTCs to humans for 1 year (S. Bettuzzi et al., 2006). No significant side or adverse effects were recorded. To our knowledge, this is the first study showing that GTCs are very effective for treating pre-malignant lesions before CaP develops. Obviously, this important achievement needs a confirmation with a larger study. But another important issue was still open, to our opinion: understanding whether CaP onset was definitively prevented or simply delayed by treatment. To deal with this issue, we performed another clinical study in which about 50% of the patients from both arms of the previous study have agreed to undergo another round of prostate mapping by needle biopsy 2ys after suspension of GTCs administration. Our follow-up study demonstrated that the incidence of CaP was still statistically different in the two arms which were previously GTCs- or placebo-treated. In fact, the clinical conditions of patients did not change after suspension of therapy, i.e. 3ys after the beginning of the study (Brausi et al., 2008). This novel data further strengthen our previous result, strongly suggesting that CaP development might be definitively inhibited in man bearing pre-neoplastic lesions by administration of GTCs for just 1y. This clinical approach can be envisaged as an early therapy which is highly effective on initial stage of cell transformation. Very interestingly, the molecular action of GTCs seems to require activation of proapoptotic, nuclear Clusterin, a novel tumor-suppressor gene (F. Rizzi and S. Bettuzzi, 2008; O. Chayka et al., 2009).

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ATTIVITÀ ANTIOSSIDANTE E ANTIPROLIFERATIVA DI PIANTE COMMESTIBILI APPARTENENTI ALLA FAMIGLIA DELLE ASTERACEAE DELLA FLORA CALABRESE

Plant drugs have a long history in both traditional and modern societies as herbal remedies or crude drugs, as purified compounds approved by Food and Drug Administration. A high number of new drugs derived from plant secondary metabolites have been applied towards in the treatment and/or prevention of cancer [1-2]. Investigations about natural products have recently regained prominence with the increasing understanding of their biological significance and increasing recognition of the origin and function of their structural diversity. Five edible plants from Calabria (Southern Italy), *Carduus pycnocephalus* L., *Cichorium intybus* L., *Cynara cardunculus* L. ssp. *cardunculus*, *Picris hieracioides* L. and *Sonchus oleraceus* L., were evaluated for their in vitro antiproliferative properties, using the Sulforodamine B (SRB) assay [3], on four human cancer cell lines: breast cancer MCF-7, prostate cancer LNCaP, amelanotic melanoma C32 and renal adenocarcinoma ACHN. After 48 h of incubation the most antiproliferative plant extract was *Cynara cardunculus* ssp. *cardunculus* on C32 and ACHN cell lines with IC_{50} of 21 and 18 $\mu\text{g/ml}$, respectively. The radical scavenging activity was assessed with DPPH test (2,2-diphenyl-1-picrylhydrazyl) [4]. The β -carotene bleaching test and the Bovine Brain peroxidation assay were used to evaluate the antioxidant activity [5-6]. The best free radical (DPPH)



scavenging activity was exerted by *Picris hieracioides*, and *Cichorium intybus* leaves extracts (IC₅₀ = 25 and 26 µg/ml, respectively). At the β-carotene bleaching test after 30 min incubation, *Picris hieracioides* extract showed the highest inhibition of linoleic acid oxidation (IC₅₀ = 3 µg/ml). Using liposomes prepared from bovine brain, *Picris hieracioides* extract, the most active ones in the previous assay, revealed the highest antioxidant effect (IC₅₀ = 22 µg/ml). In conclusion, this work reveals that the Calabrian flora, among which the species *C. cardunculus* L. ssp. *cardunculus*, *Picris hieracioides* and *C. intybus*, can be an interesting source of antioxidant and antiproliferative principles, particularly phenolic compounds and phytosterols, and a potential biomedical application in the combination therapy of cancer diseases may be suggested. This study support the various hypothesis on the fact that the Mediterranean diet is one of the healthiest dietary patterns in the world due to its relation with a low morbidity and mortality for some chronic diseases. The traditional Mediterranean diet is characterized by high consumption of foods of plant origin and relatively low consumption of red meat.

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OLI ESSENZIALI DI SALVIA BRACTEATA E SALVIA RUBIFOLIA LIBANESI: ATTIVITÀ ANTIMICROBICA E INIBITORIA DI CELLULE DI MELANOMA UMANO

The genus *Salvia*, the largest genus of the Lamiaceae family, comprises about 900 species widespread all over the world. Many *Salvia* species are used in folk medicine all around the world [1] for their antibacterial, antitumor, anti-inflammatory, antituberculosis activities, as a flavour and food condiment, in cosmetics, perfumes, for their insecticidal effect and are cultivated because of their aromatic nature [2]. Plants belonging to *Salvia* genus show high diversity in their secondary metabolites, such as flavonoids, phenolic compounds and terpenoids. This genus is also acknowledged worldwide because of the beneficial uses of the essential oils produced by the foliage. There are a number of literature reports on analyses of essential oils from plants of this genus and morphological and genetic variations are also observed according to their geographical origin. In the Lebanese folk medicine, *Salvia* spp. are used by many people in various villages and towns for the therapeutic value of their essential oil

and water extracts. Herbalists of Lebanon, Syria, and Jordan consider this species as a 'panacea' i.e. a universal drug. The plant is sold in the market; the leaves are boiled as a tea for the relief of headaches, stomachaches, abdominal pain and many other disorders [3]. *Salvia bracteata* Banks et Sol. and *Salvia rubifolia* Boiss. are two *Salvia* species well known in the traditional medicine in Lebanon. Therefore, in the continuation of our investigations on the essential oils of *Salvia* species [4], in this paper we report the composition and the biological activity of the essential oils obtained from aerial parts of *S. bracteata* Banks et Sol. and *S. rubifolia* Boiss. growing wild in Lebanon. The essential oils, extracted by hydrodistillation, were analysed by GC and CG/MS. Results showed that the major components of *S. bracteata* were caryophyllene oxide (16.6%), β-caryophyllene (4.1%), pulegone (3.9%) and terpinen-4-ol (3.8%), while in *S. rubifolia* prevailed γ-murolone (11.8%), α-pinene (7.1%), γ-cadinene (5.5%), trans-pinocarvyl acetate (5.5%) and α-thujone (5.1%). On the whole, in the oil of *S. bracteata* the monoterpene and sesquiterpene fractions were present in quite similar amounts, 33.4% and 37.1% respectively, while in *S. rubifolia* the half of the oil was constituted by sesquiterpenes. The oils were evaluated for their antimicrobial activity against ten bacterial strains finding that they were particularly active towards Gram-positive bacteria. Besides, the essential oils were tested in vitro for their potential human tumor cell growth inhibitory effect on M14 human melanoma cells. The data obtained evidenced that both essential oils were active against M14 cells. But the oil of *S. rubifolia* exhibited more potent efficacy, that could be due, at least in part, to a higher sesquiterpene content.

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CONTENUTO DI CAFFEINA E POLIFENOLI IN TÈ NERO E VERDE COMUNEMENTE USATI COME BEVANDA: INFLUENZA DEL METODO DI PREPARAZIONE

Introduction

Nowadays, there is wide knowledge on the chemical components extracted from teas. The chemical in teas is deeply influenced by the different plant cultivars and method of manufacture of tea, then the extract composition depends by several factors such as the amount of tea used, the type, amount and temperature of solvent added to the leaves, the agitation speed used to assist infusion, the length of time the leaf is left in contact with the water and the use of additional ingredients. Nevertheless, sometimes the variables investigated in literature do not properly represent the real preparation habits of tea. In this work, a contribution in understanding the chemical composition of different qualities of tea is proposed, taking into account the preparation method suggested in specialized tea shops for brewing the leaves and obtain a tea having the best flavor. Two qualities of black teas (Darjeeling and Ceylon) and four qualities of green teas (1 from China and 3 from Japan) have been analyzed. These types of teas have been selected because they represent different varieties of plants, namely *Camelia sinensis* and *Camelia assamica*, different cultivars, and different methods of manufacture. Moreover, they are also commonly used as drinking tea.

The tea sample information are reported below:

- black tea from India, Darjeeling region, plantation "Margaret's Hope", period of harvest "autumnal", full name of tea "Margaret's Hope FTGFOP1"
- black tea from Sri Lanka, Vva

region, plantation "Saint James", full name of tea "Saint James OP"

- green tea from China, Zhejiang region, full name of tea "Lung Ching"
- green tea from Japan, full name of tea "Sencha Fuji-Yama"
- green tea from Japan, Kyushu island, full name of tea "Sencha Ariake"
- green tea from Japan, full name of tea "Yamagicha", best known as "bancha green".

The aim of this work was to compare the levels of tea constituents extracted from loose-leaf teas at different temperature and infusion time by using a commercial drinking water as a solvent. To obtain the best flavor, the selected teas have to be prepared according the following procedure:

- "Margaret's Hope FTGFOP1" - water temperature: 95 °C, infusion time 3-5 min.
- "Saint James OP" - water temperature: 95 °C, infusion time 3-5 min.
- "Lung Ching" - water temperature: 85 °C, infusion time 3 min.
- "Sencha Fuji-Yama" - water temperature: 75 °C, infusion time 2-3 min.
- "Sencha Ariake" - water temperature: 75 °C, infusion time 2-3 min.
- "Yamagicha" - water temperature: 75 °C, infusion time 2-3 min.

Teas were prepared by weighting 2.5 g of the loose-leaf teas, pouring 250 mL of drinking water previously heated at each specific temperature and infusing for 3 min. The samples were filtered and immediately analyzed, to avoid degradation of catechins. Drinking water was selected as the most suitable solvent for infusion because it mimics the home procedure. Moreover, the lack of minerals in distilled water produces a bland tasting infusion. The results found after 3min infusion were compared with those obtained a) by increasing the infusion time to 10 min and b) by brewing the leaves under magnetical stirring at 0, 2 and 4 min of infusion for 30 s each. Afterward, taking into account the relatively recent increasing trend in drinking iced tea, the effects of a prolonged infusion in water at room temperature (4, 5 and 6h) were also investigated; the procedure of brewing tea in

water at low temperature for hours is emerging as one of the best method to obtain easily an iced tea which maintains its flavours for many hours.

The composition of teas varies with species, season, age of the leaf, climate and other factors. In this work the xanthine content (theobromine, theophylline and caffeine) and the major tea catechins, (-)-epigallocatechin 3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin (ECG) and (+)-catechin (C), were investigated.

Results and discussion

After 3min infusion without stirring and with water heated at the specific temperatures, gallic acid, theobromine, caffeine and catechins were extracted from black and green teas. Theophylline was never detected. Among catechins, all investigated polyphenols, namely EGCG, EGC, EC, ECG and C, were detected in the analyzed samples. Great differences were observed in the catechin contents among the three green teas from Japan. Other peaks were observed in the HPLC chromatogram, not recognized by using references standards and having a height-peak of minor interest.

The extraction efficiency of some tea components was significantly affected by the prolonged extraction time and the agitation of solvent. Even if it's not recommended for the taste of tea, the infusion time of 10 min led to highest levels of catechins, but also caffeine content was increased. Similarly, an increased amount of all constituents was measured by stirring.

Catechins and the other tea constituents were detected in the iced teas. As expected, a bigger number of unknown peaks was revealed in the HPLC chromatogram, maybe due to the increased degradation of the tea components in water. However, all the selected catechins were well separated and quantified. The extraction was not strongly influenced by the investigated infusion time in iced water.