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Phytochemicals and Nutraceuticals: the biochemical core of the Mediterranean plants

Abstract

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The Mediterranean lands are ideal sites for providing any kind of green food. In this scenery Sicily may really be considered a Treasure Island. The known benefits from Mediterranean diet, and the ever-increasing awareness of the benefits from consumption of fruits and vegetables, generated a great input in researching about typical Sicilian species and their impact on human health. We first investigated properties and bioactivity of cactus pear fruits [*Opuntia ficus-indica* (L.) Mill.] and its characteristic phytochemicals, betanin and indicaxanthin. The latter, only occurring in the cactus pear among the edible fruits, has been the object of most of our research. Chemical, physico-chemical, antioxidant and reducing properties, as well as bioactivities, from anti-inflammatory to anti-proliferative, have been described in various systems, cell cultures and animal models. Moreover, we ascertained that indicaxanthin is highly bioavailable in man, making a real link between experimental set-ups and potential activity in the body.
In other studies on bioactive polymeric proanthocyanidin components of Sicilian pistachio (*Pistacia vera* L.), we observed inhibition of the inflammatory response triggered by cytokine IL-1B in human intestinal epithelial cells, suggesting beneficial effects for the gastro-intestinal pathophysiology.
Other investigations on extracts of caper (*Capparis spinosa* L.) showed antioxidative activity of caper, including the capacity of reducing the highly reactive hypervalent-iron heme, a radical formed in red meat during cooking. In addition, the extract components from a serving size (8.6 g capers), prevented the autoxidation of meat in a simulated gastric digestion.
Finally we are studying composition, reducing power, antioxidant potential, anti-inflammatory and anti-proliferative activity of manna, the product of spontaneous solidification of the sap pouring from appropriate incisions on the bark of the trunk and main branches of two different native species of ash (*Fraxinus ornus* L. and *F. angustifolia* Vahl) cultivated in North Sicily (Madonie). In addition, we patented a method to purify, and then re-qualify the waste matter from manna remaining adherent to the bark of the tree.

Key words: Mediterranean diet, Antioxidant activity, *Opuntia*, *Pistacia*, *Capparis*, *Fraxinus*, Manna.

The known benefits from Mediterranean diet, and the ever-increasing awareness of the benefits from consumption of fruits and vegetables, generated a great input in researching about typical Sicilian species and their impact on human health.

Our Lab has been one of the first to carry out systematic investigations on properties and bioactivity of cactus pear fruits [*Opuntia ficus-indica* (L.) Mill.] and its characteristic phytochemicals, betanin and indicaxanthin. The latter, only occurring in the cactus pear among the edible fruits, has been the object of most of our research. Chemical, physicochemical, antioxidant and reducing properties, as well as bioactivities, from anti-inflammatory to anti-proliferative, have been described in various systems, cell cultures and animal models. Moreover, we ascertained that indicaxanthin is highly bioavailable in man, making a real link between experimental set-ups and potential activity in the body (Livrea & Tesoriere 2015).

Discovering the activity of dietary phytochemicals at the level of intracellular signal transduction pathways is now considered the basis to suggest their eventual health effects. The bioactivity of these molecules is generally ascribed to redox and antioxidant properties, with a growing body of evidence indicating that most compounds are to be considered for their roles as modulators of redox-mediated signaling cascades, including those relevant to either survival or cell death. Betalains are nitrogen-containing pigments occurring in the *Cariophyllales* order of plants, including beetroot and cactus pear, and in some fungal genera. Indicaxanthin (Ind, Fig.1), the yellow betalain characterising the *Opuntia ficus-indica,* has recently emerged as a radical scavenger and antioxidant with peculiar physicochemical characteristics, allowing the molecule to interact with and locate in membranes and the potential to act at the level of body cells and tissues.

Anti-inflammatory and protective effects of Ind have been shown *in vitro*, in endothelial cell cultures, in either healthy or pathological erythrocytes, and in inflammatory rat model when administered at concentrations comparable with the amounts reached in man after a dietary supplementation with cactus pear fruits. Other studies showed modulatory activity of Ind on the contractility of isolated mouse ileal muscle. Differently from the majority of

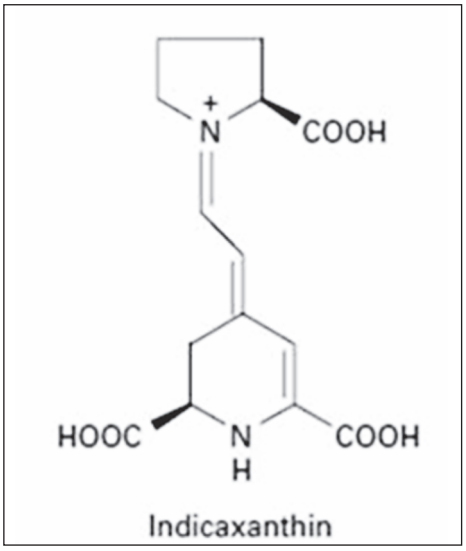

Fig. 1 Molecular structure of indicaxanthin.

dietary phytochemicals, Ind has appeared to be quite stable in absorptive gastrointestinal conditions, is not metabolized by human enterocytes or hepatocytes and is bioavailable, reaching plasma micromolar concentrations after ingestion of four fruits (Tesoriere & al. 2013).

A strong, reciprocal crosstalk between inflammation and melanoma has rigorously been demonstrated in recent years, showing how crucial is a pro-inflammatory microenvironment to drive therapy resistance and metastasis. We investigated on the effects of Indicaxanthin against human melanoma both *in vitro* and *in vivo*. The effects of indicaxanthin were evaluated against the proliferation of A375 human melanoma cell line and in a mice model of cutaneous melanoma. Cell proliferation was assessed by MTT assay, apoptosis by Annexin V-Fluorescein Isothiocyanate/Propidium Iodide staining, protein expression by western blotting. Melanoma lesions were subcutaneously injected in mice with B16/F10 cells, chemokine release was quantified by ELISA. We found that indicaxanthin effectively inhibits the proliferation of the highly metastatic and invasive A375 cells as shown by growth inhibition, apoptosis induction and cell invasiveness reduction. More interestingly, the *in vitro* data were paralleled by those *in vivo* showing that indicaxanthin significantly reduced tumor development when orally administered to mice. The results of our study also clarify the molecular mechanisms underlying the antiproliferative effect of indicaxanthin, individuating the inhibition of NF-kB pathway as predominant. In conclusion, we demonstrated that indicaxanthin represents a novel phytochemical able to significantly inhibit human melanoma cell proliferation *in vitro* and to impair tumor progression *in vivo*. When considering the resistance of melanoma to the current therapeutical approach and the very limited number of phytochemicals able to partially counteract it, our findings may be of interest to explore indicaxanthin potential in further and more complex melanoma studies in combo therapy, i.e. where different check points of melanoma development are targeted (Allegra & al. 2019).

In other studies on bioactive polymeric proanthocyanidin components of Sicilian pistachio (*Pistacia vera* L.), we observed inhibition of the inflammatory response triggered by cytokine IL-1β in human intestinal epithelial cells, suggesting beneficial effects for the gastro-intestinal pathophysiology. Intestinal epithelial cells (IECs) have a strategic position at the interface between the antigenic luminal environment and the internal milieu, and play an important role in the enteric immune system by establishing bidirectional interaction with the underlying immune cells. As a real physical barrier, IECs can modulate a direct exposure of immune cells to antigens and can initiate a transient inflammatory response with the secretion of inflammatory mediators. Inflammation of IECs to a moderate extent is normal and crucial to maintain gut structural integrity and function. On the other hand, alteration and dysregulation of inflammatory pathways can increase the number and activity of inflammatory cells in the gut mucosa, and substantially contribute to the pathogenesis of IBD. As the therapeutic approaches to IBD are not entirely satisfactory, the importance of prevention cannot be overlooked. In this context, the influence of dietary components on the mucosal inflammatory processes may be relevant. Plant polyphenols, in particular flavonoids, are one example of these compounds, and proanthocyanidins are a subgroup of flavonoids exhibiting potential biological actions, including anti-inflammatory activity. Data from human subjects show that high molecular weight proanthocyanidins - with more than three subunits - do not undergo intestinal absorption. However, beneficial effects of these dietary components on the digestive tract may not require efficient

absorption through the gut. Rather, due to their high digestive stability and their poor intestinal absorption, these compounds could achieve high luminal concentrations thus meeting the potential conditions to participate in reactions relevant to digestive tract health through direct interaction with the IECs. In this context, plant extracts with high proanthocyanidin content have shown anti-inflammatory and protective effects in animal models of chronic intestinal inflammation, such as ulcerative colitis and inflammation-associated colorectal cancer. The genus *Pistacia* contains only 11 species among which *Pistacia vera* L. is the sole producing edible nuts. Experimental data show that the pistachio nut consumption has positive effects on human serum lipid profile and cardiovascular disease (CVD) risk factors and significantly improves oxidative status and reduces circulating inflammatory biomarkers. On the other hand, anti-inflammatory effects of pistachio nut are still poorly characterized from a molecular point of view.

Previous research from our group provided evidence that a hydrophilic extract from Sicilian pistachio nuts (HPE) contains substantial amounts of polyphenols, including proanthocyanidins, and possesses radical-scavenging and anti-oxidative properties. Moreover, we also demonstrated that HPE has anti-inflammatory activities in LPS activated macrophages, interfering with the NF-kB activation, and that its high molecular weight proanthocyanidins can play a major role as bioactive component of HPE. Dietary approaches to control inflammatory bowel diseases (IBD) may include proanthocyanidin-rich foods. We then studied the effects of HPE and of its polymeric proanthocyanidin fraction (PPF) in a cell model that simulated some conditions of IBD, consisting of interleukin (IL)-1β-stimulated Caco-2 cells. HPE was prepared by *Pistacia vera* L. nuts, and PPF was isolated from HPE by adsorbance chromatography. Proanthocyanidins were quantified as anthocyanidins after acidic hydrolysis. Differentiated Caco-2 cells were pre-incubated with HPE or PPF and then were exposed to IL-1β. Cell viability and parameters associated with nuclear factor-kB (NF-kB) activation were assayed. Adsorption of polymeric proanthocyanidins to the cell membrane was investigated by transepithelial electrical resistance (TEER) measurements. HPE decreased prostaglandin (PG)E_2 production, IL-6 and IL-8 release, and cyclooxygenase (COX)-2 expression. HPE also inhibited the increase in paracellular permeability and reduced NF-kB activation. Polymeric proanthocyanidins, tested at a concentration comparable with their content in HPE, produced effects comparable to HPE. Finally, cell exposure to PPF increases TEER of the epithelial monolayers. Our results provide evidence that pistachio nut components inhibit inflammatory response of intestinal epithelial cells *in vitro* and indicate polymeric proanthocyanidins as the major bioactive nut components. The protection implies inhibition of NF-kB activation and occurs in parallel with the adsorption of polymeric proanthocyanidins to cell membrane. Our findings suggest that intake of small amounts of pistachio nut can exert beneficial effects to gastrointestinal pathophysiology (Gentile & al. 2012, 2014).

Other investigations on extracts of caper (*Capparis spinosa* L.) showed antioxidative activity of caper, including the capacity of reducing the highly reactive hypervalent-iron heme, a radical formed in red meat during cooking. In addition, the extract components from a serving size (8,6 g capers), prevented the autoxidation of meat in a simulated gastric digestion.

Capparis spinosa L. is one of the most common aromatic plants growing wild in the dry regions around the Mediterranean basin. In Italy, this blue-gray spiny plant is intensively

cultivated, particularly on small islands around Sicily, such as Pantelleria and the Eolian Salina, which provide 95% of the national product. Capers are appreciated for their pungent and bitter flavor and are used as an appetizer with olives, cheese, and nuts or as a complement to meat, salads, pasta, and other foods. The aromatic floral buds are gathered just before blooming and stabilized either in brine or in salt. During this processing the plant glucosinolates are converted to their cognate isothiocyanates, responsible for the characteristic flavor of the caper, in a concomitant fermentation process. The fermentation is completed within 30 days, and edible capers are usually marketed after 2–3 months. Apart from its use as flavoring, the caper has been known for centuries in traditional phytomedicine, which exploited its properties for several purposes. The aqueous extract from total aerial parts of the plant has been used for its antifungal, anti-inflammatory, antidiabetic, and antihyperlipidemic activities and is among the constituents of polyherbal formulations to treat liver ailments. Other investigations showed that raw floral buds contain lipids, alkaloids, glucocapperin as major glucosinolate, and a number of antioxidant phytochemicals such as flavonoids and other polyphenols. Quercetin-3-rutinoside (rutin) has appeared to be the most abundant flavonoid in *C. spinosa*.

Floral buds of *Capparis spinosa* L. are commonly used in the Mediterranean cuisine as flavoring for meat and other foods. We evaluated bioactive components and antioxidant activity of Sicilian capers from Pantelleria (Sicily) stabilized in salt. Whereas R-tocopherol was absent, low levels of γ-tocopherol and vitamin C were measured. With reference to one serving size (8.6 g of capers), rutin was 13.76 mg, isothiocyanates, recently acknowledged as anticarcinogen phytochemicals, were 42.14 μmol, total phenols were 4.19 mg of gallic acid equivalents (GAE), and the total antioxidant potential measured using the [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt (ABTS) cation radical decolorization assay was 25.8 μmol of Trolox equivalents. The antioxidative activity of a caper hydrophilic extract was assessed in a number of assays. The extract at 3.5 and 7.0 μM GAE exhibited a dose-dependent peroxyl radical scavenging activity in a methyl linoleate methanol solution oxidized by azo initiator, and reduced hypervalent iron myoglobin species formed from met-Mb and H_2O_2, at 180 μM GAE. The hydrophilic extract, at 70–280 μM GAE, caused a dose-dependent inhibition of lipid autoxidation in heated red meat, incubated with simulated gastric fluid for 180 min. In the same model rutin tested at a concentration corresponding to its content in the extract was ineffective, and R-tocopherol at 25 μM was poorly effective. The hydrophilic extract (70 μM GAE) prevented the consumption of the co-incubated R-tocopherol, whereas lipid oxidation was inhibited for the experimental time, suggesting cooperative interactions between extract components and the vitamin. The findings encourage the use of caper with foods that contribute oxidizable lipids in view of the association between dietary oxidized lipids and risk of oxidative stress-based diseases (Tesoriere & al. 2007).

Finally we are studying composition, reducing power, antioxidant potential, anti-inflammatory and anti-proliferative activity of the manna.

Known for centuries within the ethnobotanical field, the Sicilian *manna* is a very singular vegetable product. It is obtained during summertime, by the spontaneous solidification of the sap pouring from incisions done, according to traditional methods, on bark and main branches of some species of the genus *Fraxinus*. The production of manna requires a number of special conditions. The trees need to be grown in areas characterized by high tem-

peratures, low humidity and low temperature range. Some areas of the Madonie Mountains in Northern Sicily are ideal pedoclimatic niches for the cultivation of the *Fraxinus* species, from which the sap is extracted and gathered using traditional methods. The manna from *Fraxinus* is available in thin cylindrical forms of whitish color (the "cannolo" manna) with a sweet and pleasant taste. In addition, we patented a method to purify, and then re-qualify the waste matter from manna remaining adherent to the bark of the tree.

The invention consists in a procedure allowing a total purification of waste matter from manna, in order to re-qualify this now neglected and undervalued material and obtain a final product with a high market price (10-15-fold the current one). The product so obtained contains all components of pure manna, and results further enriched with phenol bio-compounds known for health-promoting properties. The purified product may be utilized in the alimentary industry, and applied in pharmaceutical and cosmetic preparations.

Sugars, mono- and oligo- saccharides make up more than 80% of the dry weight of the *Fraxinus* manna. Among them mannitol is the main constituent, representing 50% of the total sugars. Therefore, health benefits of manna have mostly been ascribed to, and are consistent with, the properties of mannitol, a polyol involved in a wide range of physiological processes in plants as regulator of the pool of cellular reductants, as a cryoprotectant, osmotically active solute and as a hydroxyl radical scavenger. On the other hand, recent phytochemical characterization of the *Fraxinus* manna has revealed a fatty acid and phenol compounds profile comparable to that of most extra-virgin olive oils (Caligiani & al. 2013), which is consistent with the chemotaxonomic closeness between *Fraxinus* and *Olea* genera. The chemistry of these components suggests that, beyond the well known physiological effects due to the presence of mannitol, the manna may provide a reducing and antioxidant potential contributing to the redox homeostasis.

Despite the widespread use of the manna in the folk medicine, little or no scientific study addressed a systematic investigation on the reducing and antioxidant properties of the manna. We used two solvents, methanol and ethyl acetate, to extract and carry out a quali-quantitative analysis of phytochemical components of manna from *Fraxinus* ssp. grown in the Madonie mountains and investigated reducing and antioxidant properties of the extracts utilizing well established chemical and biological screening methods (manuscript in preparation).

The Mediterranean is one of the ideal places for providing any kind of green food. In particular Sicily may really be considered a Treasure Island. Sicilian fruits and vegetables, and their bioactive phytochemicals, are currently studied intensely for basic science and applied research. Understanding how these compounds exert physiological effects when ingested with food and exploiting the properties of the nutraceutical components is the key goal of our study of molecular nutrition.

References

Allegra, M., De Cicco, P., Ercolano, G., Attanzio, A., Busà, R., Cirino, G., Tesoriere, L., Livrea, M. A. & Ianaro, A. 2019: Indicaxanthin, a multi-target natural compound from *Opuntia ficus-indica* fruit: From its poly-pharmacological effects to biochemical mechanisms and molecular modelling studies. – Europ. J. Med. Chem. **179:** 753-764. http://dx.doi.org/10.1016/j.ejmech.2019.07.006.

Caligiani, A., Tonelli, L., Palla, G., Marseglia, A., Rossi, D. & Bruni, R. 2013: Looking beyond sugars: Phytochemical profiling and standardization of manna exudates from Sicilian *Fraxinus excelsior* L. – Fitoterapia **90:** 65-72. http://dx.doi.org/10.1016/j.fitote.2013.07.002

Gentile, C., Perrone, A., Attanzio, A., Tesoriere, L., Livrea, M. A. 2015: Sicilian pistachio (*Pistacia vera* L.) nut inhibits expression and release of inflammatory mediators and reverts the increase of paracellular permeability in IL-1-exposed human intestinal epithelial cells. – Eur. J. Nutr. **54(5):** 811-821. http://dx.doi.org/10.1007/s00394-014-0760-6

—, Allegra, M., Angileri, F., Pintaudi, A. M., Livrea, M. A., Tesoriere, L. 2012: Polymeric proanthocyanidins from Sicilian pistachio (*Pistacia vera* L.) nut extract inhibit lipopolysaccharide-induced inflammatory response in RAW 264.7 cells. – Eur J Nutr. **51(3):** 353-363. http://dx.doi.org/10.1007/s00394-011-0220-5

Livrea, M. A., Tesoriere, L. 2015: Indicaxanthin dietetics: past, present and future.– Pp. 141-163 in: Chunxian, C. (ed.), Pigments in fruits and vegetables. Genomics and dietetics. – Lake Alfred.

Tesoriere, L., Butera, D., Gentile, C. & Livrea, M. A. 2007: Bioactive components of caper (*Capparis spinosa* L.) from Sicily and antioxidant effects in a red meat simulated gastric digestion. – J. Agric. Food Chem. **55(21):** 8465-71. http://dx.doi.org/10.1021/jf0714113

—, Attanzio, A., Allegra, M., Gentile, C. & Livrea, M. A., 2013: Phytochemical indicaxanthin suppresses 7-ketocholesterol-induced THP-1 cell apoptosis by preventing cytosolic Ca(2+) increase and oxidative stress. – Br. J. Nutr. **110:** 230-240. http://dx.doi.org/10.1017/S000711451200493X

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