

## IL PANE DELLE DONNE®

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The female life is closely related to hormonal changes that begin at puberty, fluctuate during the reproductive age and dramatically decline in menopause, a condition with which ends the production of oestrogens by the ovary. Oestrogen has a crucial role in the control of various physiological activities such as bone turnover, cardiovascular system, brain, connective system, protein and glucose metabolism, and many others. As age increases, the hormonal level decreases, causing a real climacteric syndrome characterized by hot flashes, night sweats, dizziness, vaginal dryness, apathy, nervousness, insomnia, weight increase, with major discomfort in social life. Symptoms may be contrasted with the use of hormone replacement therapy or phytoestrogens, in particular the soy isoflavones widely documented to be able to counteract most of the clinical conditions present in menopausal women. Il Pane delle Donne® is a handmade product that can provide

the daily dose of phytoestrogens recommended for typical symptoms of menopause. It was realized in 2014 thanks to the collaboration between researchers of Siena University and craftsmen, this food contains only whole raw materials that enrich the bread with their constituent molecules. The loaves are present on the market in pieces of 200 g and 500 g and contain about 20 - 25 mg of isoflavones per 100 g of product, together with good amount of protein and fiber and small amount of carbohydrates. The development of this product was based on the conviction that the bread, common food consumed daily, can be the right vehicle to introduce the bioactive molecules in the diet of women in menopause. Preliminary research in our departments already showed the ability of the regular consumption of this bread, to contrast the menopausal symptoms.

**Key words:** Phyto-oestrogen, isoflavones, soy food, menopause.

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## THE POLLEN TUBE AS A MODEL SYSTEM FOR THE STUDY OF PLANT CELL MORPHOGENESIS

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The pollen tube is a fundamental cell in the reproductive process of higher plants. The evolution of this cell allowed plants to be independent of water for both transport and fusion of gametes. In this way, plants were able to invade lands by gradually adapting to live in conditions of water scarcity. The pollen tube is a cell that grows in an extremely polarized way through female tissues in order to complete the fertilization process. The growth of pollen tubes is a complex process that requires the synchronized activity of many different factors. In flowering plants, pollen tubes can grow through relatively

solid tissues of the pistils. To carry out this process, pollen tubes require a well-adapted shape consisting of a tubular shaft ending with a hemispherical dome. In order to maintain such shape, pollen tubes must build a highly dynamic cell wall that is perfectly adapted to the cell's penetrating activity. Therefore, the molecular mechanism that controls the architecture of pollen tubes is critical. In growing pollen tubes, the cytoskeleton controls the intracellular transport of organelles and vesicles. The movement of these membranous structures is necessary for apical growth of pollen tubes and then for the correct assembly of the cell wall. This process is closely related to the precise deposition of specific proteins and polysaccharides, which contribute to local differentiation of cell wall structures and therefore to the tip-growth mechanism of pollen tubes. Considering the peculiar temporal and spatial arrangement of the cell wall, the pollen tube is now considered an excellent model for studying plant cell morphogenesis.

**Key words:** morphogenesis, cell wall, cytoskeleton, pollen, plant cell.

## CALCULATING EXAPTATION AS QUANTITY

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Exaptation can be calculated basing it on numerical parameters. The model is based on and combine indicators regarding

persistence of structural-functional features and *variation* of ordinary function. A quantitative descriptor (as per cent value) is assigned to each chosen parameter and it is inserted in the mathematical model.

*Persistence indicators* are: morphology, relative dimensions, metabolic/biochemical functionality, spatial relation with other organs/apparatuses/systems, histologic/cytological features, development/maturation, genetics. *Variation indicators* are assembled in some main areas: mechanical (statics and dynamics), thermal, perceptual, informational, biochemical,

reproductive, self-preservation, predation and nutrition. Along the range of variability of the value of the exaptation, we can establish a limit to distinguish exaptation from *no-exaptation*. To detect exaptation (E), we must have both persistence of structure (A) and variation of its function (B), so that:  $(A) \cap (B) \rightarrow E$ . Besides only if the value of the product of  $(A) \cdot (B)$

overcomes a limit P, we have exaptation E:  $[(A) \cdot (B) \geq P] \rightarrow E$ . We will indicate an hypothesis of implementation of this model: a research plan for the specific case of human rachis.

**Key words:** exaptation, calculating, parameter, mathematical function.

### ANTI-INFLAMMATORY PROPERTIES OF THE H<sub>2</sub>S DONOR DRUG ZOFENOPRILAT ON CELLS OF THE VASCULAR WALL

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Cardiovascular diseases as atherosclerosis are associated to an inflammatory state of the vessel wall which is accompanied by endothelial dysfunction, and adherence and activation of circulating inflammatory cells. Hydrogen sulfide, a novel cardiovascular protective gaseous mediator, has been reported to exert anti-inflammatory activity. We have recently demonstrated that the SH containing ACE inhibitor zofenoprilat, the active metabolite of zofenopril, controls the angiogenic features of vascular endothelium through H<sub>2</sub>S enzymatic production by cystathionine gamma lyase (CSE). Based on H<sub>2</sub>S donor/generator properties of zofenoprilat, the objective of this study was to evaluate whether zofenoprilat exerts anti-inflammatory activity in vascular cells.

Here we found that zofenoprilat, in a H<sub>2</sub>S-mediated manner, abolished all the inflammatory features induced by interleukin-1beta (IL-1β) in human umbilical vein endothelial cells (HUVEC), especially the NF-κB/cyclooxygenase-2 (COX-2)/prostanoid biochemical pathway. The pre-incubation with zofenoprilat/CSE dependent H<sub>2</sub>S prevented IL-1β induced paracellular hyperpermeability through the control of expression and localization of cell-cell junctional markers ZO-1 and VE-cadherin. Moreover, zofenoprilat/CSE dependent H<sub>2</sub>S reduced the expression of the endothelial markers CD40 and CD31, involved in the recruitment of circulating mononuclear cells and platelets. Interestingly, this anti-inflammatory activity was also confirmed in vascular smooth muscle cells and fibroblasts as zofenoprilat reduced, in both cell lines, proliferation, migration and COX-2 expression induced by IL-1β, but independently from the SH moiety and H<sub>2</sub>S.

These in vitro data document the anti-inflammatory activity of zofenoprilat on vascular cells, reinforcing the cardiovascular protective effect of this multitasking drug, which can be now considered a H<sub>2</sub>S donor/generating compound.

**Key words:** endothelial cells, hydrogen sulfide, inflammation, interleukin-1, smooth muscle cells.

### VENOUS LEG ULCERS INFECTED BY STENOTROPHOMONAS MALTOPHILIA

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#### Introduction

*Stenotrophomonas maltophilia* is a ubiquitous bacterium, aerobic, non-fermentative, belonging to the gram-negative group, closely linked to *Pseudomonas* specie. It is only species of *Stenotrophomonas* known to infect humans beings and it is an opportunistic pathogen typical in debilitated patients. In recent years, the infection from *Stenotrophomonas maltophilia* is becoming more common due to both increase of isolations of this bacterium along with an increase of cultures in hospitalized sick people and increase of patients with risk factors.

#### Case report

A 48 years old man came to our attention for the presence of venous ulcers and lower legs oedema. The patient's his-

tory reveals the presence of right hemiparesis, psycho-intellectual deficit with behavioural disorders, epilepsy, surgical operation in 2006 for bowel obstruction after appendectomy and again in 2007 for viscerolysis and cholecystectomy. The patient has been suffering from these ulcers for 10 years and he was followed in different hospitals in Tuscany. He was always treated with broad-spectrum antimicrobial therapy and compression bandages with resolution of the lesion. The ulcers recurred, unfortunately, after discontinuation of antibiotic therapy. A bacterial culture performed at our microbiology laboratory highlighted the presence of *Stenotrophomonas maltophilia*. Blood testing on routine basis was performed and additional specific testes were carried out in a second step to exclude HIV infection. A duplex scan of lower limbs does not show any pathology of the arterial side, while at the venous level showed a great saphenous vein insufficiency with truncal reflux up to the lower third of the left leg. The ulcers were treated locally with advanced medications and bandages. Then after results of antibiotics susceptibility testing the therapy with Trimethoprim/Sulfamethoxazole 1 cp × 2 daily has been established for three weeks. At the end, it was repeated the buffer with culture test that confirmed to be negative. The ulcer lesion of patient is currently in slow but progressive increase.

**Discussion**

A multicentre study performed in the US has put *Stenotrophomonas maltophilia* eleventh place as the most frequent microorganism in Hospitals. This bacterium is a germ that is expanding seen at our centre on 31 cultures taken in a year this is already the second, equal to 6% of the total. If we take into consideration the potential danger of this bacterium in patients with multiple illnesses, the elderly with complex drug therapies as are patients who flock to our clinics we can see the need for continuous monitoring of the bacterial flora to prevent first evolution up to paintings by cellulite, gangrenous cellulitis, skin necrosis and ecthyma gangrenosum and mucocutaneous ulcers to more severe forms such as urinary tract infections, respiratory, endocarditis, bacteraemia, septicaemia. Considering also that *Stenotrophomonas maltophilia* is a gram-negative bacterium that can survive virtually ubiquitous in all wet areas (including floors and water plants) we understand how important it is to maintain a high state of alert and is easy to inadvertent contamination of other patients. It is therefore

of particular importance the prevention of infection by *Stenotrophomonas maltophilia*, which can be obtained through education of health personnel to prevent the transmission and spread of the bacterium, the regular cleaning and disinfection of surfaces and medical instruments, the routine washing of hands, the regular maintenance of hospital equipment and replacement of defective and worn parts, and the targeted use of antibiotics in order to limit antimicrobial resistance and the spread of the organism.

**Conclusions**

In the case of our patient the contamination and the onset of symptoms it is certainly due to the long-term treatment with broad-spectrum antibiotics and some contact with the source occurred in one of several hospital admissions seen that among the factors predisposing to infection are recognized in addition to a prolonged hospitalization especially in intensive care units, bladder catheterization, immunodeficiency.

**Key words:** *Stenotrophomonas maltophilia*, venous leg ulcers.

**ANTIOXIDANT AND NUTRACEUTICAL PROPERTIES OF TOMATINE AND ITS DERIVATIVES**

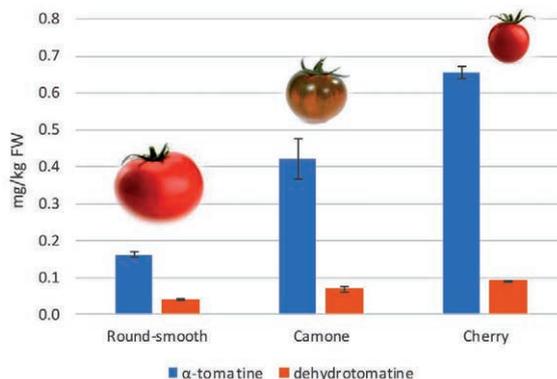
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In the present work, the nutraceutical properties of tomato fruits (*Solanum lycopersicum* L.) were investigated. In particular, the attention was focused on the study of tomatine, a glycoalkaloid synthesized by tomato plants and green tomatoes as a defense against the adverse effects of pathogens as fungi and bacteria. Indeed, the tomato glycoalkaloid referred to as tomatine is composed by a 10:1 mixture of  $\alpha$ -tomatine and dehydrotomatine. Tomatoes represent a source of other bioactive compounds, as polyphenols, important for their antioxidant properties and their ability to reduce the risk of triggering of chronic diseases, like some types of cancers and cardiovascular diseases.

Firstly, the whole fruits of three tomato varieties were analysed: round-smooth, cherry, and camone tomatoes. Camone tomato is an Italian variety especially cultivated in Sicily and Sardinia which remains green in the upper part of the fruit also at the mature stage of ripeness. Quantification of  $\alpha$ -tomatine and dehydrotomatine in all the samples was performed by reverse phase liquid chromatography coupled with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) on a triple quadrupole analyser. The two glycoalkaloid concentrations found in camone tomatoes

( $\alpha$ -tomatine=0.442 $\pm$ 0.055; dehydrotomatine=0.069 $\pm$ 0.008 mg/kg FW, fresh weight) were intermediate with respect to the amount found in round-smooth tomatoes ( $\alpha$ -tomatine=0.162 $\pm$ 0.007; dehydrotomatine=0.039 $\pm$ 0.001 mg/kg FW) and cherry tomatoes ( $\alpha$ -tomatine=0.655 $\pm$ 0.019; dehydrotomatine=0.091 $\pm$ 0.001 mg/kg FW; Figure 1).



**Figure 1.** Contents of  $\alpha$ -tomatine and dehydrotomatine in three different tomato varieties.

Subsequently, different parts of camone tomato fruit were studied, and analyses were carried out on peel, pulp and locular gel. The concentrations of  $\alpha$ -tomatine and dehydrotomatine in the locular gel were 3.87 $\pm$ 0.33 mg/kg FW and 0.490 $\pm$ 0.001 mg/kg FW, respectively, and they resulted about ten times lower in the peel, and were not detected in the pulp. For all the samples, total polyphenols content was evaluated by using the Folin-Ciocalteu assay, while the ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) and DPPH (di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium) assays were used to measure the antioxidant activity as Trolox Equivalent Antioxidant Capacity (TEAC). Individual polyphenols were quantified by using HPLC-

ESI-MS/MS. The results showed that chlorogenic acid and caffeic acid were the main hydroxycinnamic acids in all the varieties, while rutin was the most abundant flavonoid. Rutin occurred mainly in the peel of the camone variety, while chlorogenic acid was primarily in the locular gel. On

the basis of these preliminary results, spontaneously hypertensive rats (SHR) were fed daily with locular gel obtained from camone tomatoes, to evaluate its efficiency as a potential nutraceutical product endowed with antihypertensive activity.

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**MICROBIOLOGICAL STUDIES OF  
ARCHAEOLOGICAL SEEDS (2000 YEARS OLD)  
CONTAINING WELL-PRESERVED MUMMIFIED  
EMBRYOS**

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At San Giovanni (Portoferraio harbour) on Elba Island, was discovered a villa from the Roman republican period (2nd-1st century BC). The foundations of the five rectangular areas walls were excavated. Palaeobotanical analysis of a set of stratigraphic layers showed pollens and spores produced by coeval vegetation with some arboreal and herbaceous pollen. Abundant microscopic particles of charcoal as indicator of area prone to fire and less human impact and supported the

hypothesis that the building was abandoned after a fire. The compact sterile layer caused by the fire formed a seal over the underlying four amphorae containing archaeological apple seeds. Morphological observations of the shape and size of the archaeological seeds provided a detailed view of rounded integuments caused by conservation in a wet environment and suggesting the samples came from four amphorae used for cider production. The 80% of the seeds showed the integument opened while 20% had a well-preserved embryo, where we have isolated and revived one fungal species and sporogenic bacteria. The fascinating hypothesis is that as we have revived the spores of fungi and bacteria maybe we could revitalize the embryos of the mummified seeds collected in Roman amphorae.

**Key words:** Archaeological seeds, Microbiological study, mummified embryos.

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