III Congresso Nazionale

della Società Italiana per la Ricerca sugli Oli Essenziali (S.I.R.O.E.)

Co-organizzato da

Associazione Volontari per Policlinico Tor Vergata o.n.l.u.s.

Roma, 6-7-8 novembre 2015

Palazzo Santa Chiara – Piazza Santa Chiara, 14

ABSTRACTS DEGLI INTERVENTI IN INGLESE

"AROMATIC" FLOWER DECORATIONS IN THE ITALIAN PAINTINGS OF THE FIFTEENTH CENTURY

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The interest in flower plants has remote roots in European culture. To discover the charm and beauty of flower decorations walk through the Italian paintings of the fifteenth century, when roses, lilies and wild flowers – many of which are now indicated as aromatic – were wonderfully inserted in texts, frescoes and oil paintings. In Ferrara, the *Libro delle composizioni vocali* (book of vocal compositions) is full of flowers adorning the musical staves. Pisanello starts the practice of painting flowers for their symbolic meaning. In Florence, Lippi inserts roses and lilies in almost all his paintings. Botticelli, in the *Adoration of the Magi*, paints *Parietaria* and *Umbellicus rupestris* and many are the existing flowers in the *Primavera*, minutely represented with a specific symbolism (buttercup for death, lily for love, chrysanthemum for virginity, jasmine for grace, etc.).

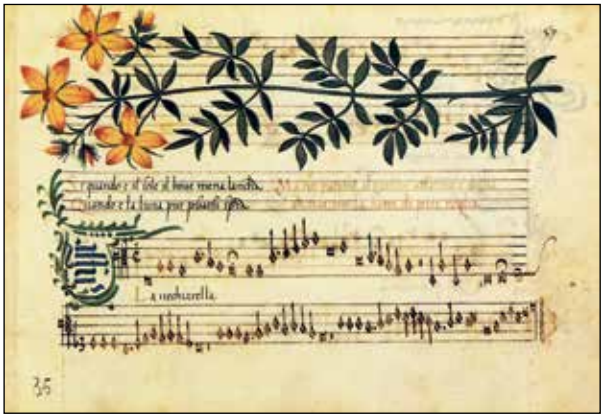

Fig. 1. A page from the "Libro delle composizioni vocali" (dating back to 1496 approximately), decorated with a sprig of St. John's wort.

Fig. 2. Antonio Pisano, "Portrait of a Princess". Roses and columbines in the background represent the aristocracy and the princess's premature death.

ABSTRACT

APPLICATION OF ESSENTIAL OILS IN CULTURAL HERITAGE: STATE OF THE ART

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The biodeterioration control of cultural heritage materials includes possible measures to stop the degradation caused by both microorganisms and organisms and, when possible, to delay its reappearance. All artefacts, with time, are attacked by biodeteriogens (BDs). The stone colonization follows a precise pattern: algae, mosses and lichens before, mushrooms and common organisms (from cryptogams to higher plants) after. Furthermore, mushrooms represent both a serious danger to the artefacts and a risk to human health. From the above, it becomes necessary to preserve both the artefacts and human health with effective methods against BDs. Despite the great use of chemical biocides, natural products, particularly essential oils, are gradually becoming effective solutions for the restoration and conservation of cultural heritage.

RELATIONSHIP BETWEEN ENVIRONMENT AND ESSENTIAL OIL YIELD OF MEDICINAL PLANTS

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The greater demand for products derived from medicinal plants is leading to the necessary revival of their cultivation in a suitable environment, using appropriate farming techniques to ensure quality production. Research conducted in Molise, on a crop of chamomile in different experimental sites, has shown that the cultivation environment, in addition to changing the production per unit area in flower heads, modified the amount of essential oil and its composition. In particular, the plants grown in higher and fertile environments produced more oil than those raised in the plains with the same strategy crop. Moreover, switching from the plain to high hill decreases the amount of cis-tonghaosu and increases that of bisabolol, which are the two main constituents of the essential oil of chamomile.

PINUS PINEA ESSENTIAL OILS AS BIOCHEMICAL SHIELD AGAINST INFECTION BY *HETEROBASIDION IRREGULARE*, AN INVASIVE PATHOGEN FUNGUS FROM NORTH AMERICA

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Heterobasidion annosum (Fr.) Bref is a native pathogen that causes root and butt-rot disease mainly on Italian stone pine (*Pinus pinea*) along the peninsula.
Heterobasidion irregulare Garbel. & Otrosina, American vicariant, was introduced in Italy during the World War II from North America and now is slowly spreading in several *P. pinea* stands along the Tyrrhenian coast.
The aim of this study was to investigate the impact of *H. irregulare* on essential oil terpenoids and ecophysiology of Italian stone pine (*Pinus pinea* L.)
For this purpose, an inoculation experiment was carried by using 3-years-old Italian stone pine seedlings in each of the following basal stem treatments: (1) *H. irregulare,* (2) *H. annosum*, (3) wounding without inoculation, (4) unwounded plants.
Variation in photosynthesis and stomatal conductance were detected in response to the attack by Heterobasidion species.
Fungal infections strongly affected the production of volatile terpenoids: differences in the relative proportions (percentages) of constitutive and induced terpenes with *Heterobasidion* spp. were observed.
Stem base inoculation with *Heterobasidion* spp. induced changes in total terpenoids contents also at a systemic level.
In general, our measurements did not show differences in the response of Italian stone pine seedlings to the invasive North American *H. irregulare* and the native European *H. annosum*.

THE ESSENTIAL OIL OF *THYMBRA CAPITATA* (L.) CAV. AS A POSSIBLE ALTERNATIVE FOR BOTH WEED AND SCAB CONTROL CAUSED BY *FUSICLADIUM ERIOBOTRYAE* (CAV.) SACC. IN THE LOQUAT CULTIVATION [*ERIOBOTRYA JAPONICA* (THUNB.) LINDL.]

Mercedes Verdeguer and Mónica Berbegal

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Loquat (Rosaceae) is an evergreen subtropical fruit. Spain is the first producer in the Mediterranean area, and the leading exporter worldwide. Medlar mottling, caused by *Fusicladium eriobotryae*, is the main disease affecting the crop and is con-

trolled by different fungicides. The new European legislation regarding pesticides has reduced their availability. The emergence of resistance in many pests is also concerning. Weed control in loquat is important for the survival of young trees. In adult trees, "non-tillage" and chemical weed control are often used. Plant essential oils (EOs) have shown insecticidal, herbicidal and antifungal properties. This work, financed by Generalitat Valenciana (GV/2014/039), tests the potential of *T. capitata* EO as an alternative for loquat crop protection.

Figgs. 3, 4: Symptoms induced by Fusicladium eriobotryae on fruits and leaves of Eryobotrya japonica.

ESSENTIAL OILS VAPOURS IN HYPOBARIC STORAGE TO CONTROL POSTHARVEST DECAY OF TABLE GRAPES

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Synthetic fungicides are commonly used means to control grey mould, the main postharvest disease of table grapes. Recently, environmental low-impact alternatives, such as the exposure to essential oil vapours, have gained attention among researchers as alternatives to prolong fruit storage. In this work, the effectiveness in controlling table grapes postharvest decay of vapours of essential oils extracted from *Rosmarinus officinalis,* Mentha piperita, and *Thymus vulgaris* was tested under atmospheric and hypobaric pressures. Among the tested essential oils, rosemary was the most effective in reducing postharvest decay of table grapes. However, untrained panellists perceived the presence of essential oils when a sensory evaluation of treated table grapes was carried out.

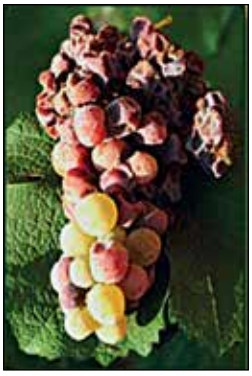

Fig. 5. Botrytis cinerea infecting a bunch of grapes.

PRETREATMENT OF *ACTINIDIA DELICIOSA* PLANTS WITH THE ESSENTIAL OIL OF *MONARDA DIDYMA*: EFFECT ON THE DISEASE CAUSED BY *PSEUDOMONAS SYRINGAE* PV. ACTINIDIAE

Paola Minardi[1], Francesco Epifano[2], Giulio Zama[3], Maria Grazia Bellardi[3]

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Plant pathogenic bacteria have recently caused severe outbreaks in economically important crops, such as *Pseudomonas syringae* pv. *actinidiae* (Psa), which causes the bacterial canker of kiwifruit. Since the control measures of bacterial diseases are essentially preventive, the use of natural substances, such as essential oils (EOs), is becoming widespread. In 2013, the antimicrobial effectiveness of the EO from *Monarda* spp. against Psa was shown *in vitro*. Based on these findings, it was hypothesized that pretreatment of *Actinidia deliciosa* plants with the EO from *M. didyma* flowering plants might subsequently interfere with or inhibit the Psa growth. On 25 May 2015, 150 micropropagated plants were subdivided into 4 groups corresponding to 4 treatments (sterile distilled water, EO 0.3%, hydrolate 14.7%, and streptomycin 100 mg/l) and after 24h they were inoculated with a bacterial suspension of the virulent Psa IPVBO 8101 strain (3 x 10^7 CFU/ml). The plant health was daily checked for damages caused by each treatment itself, and for the symptoms induced by Psa. At the end of July, all leaves were collected to determine the number of spots/leaf/plant for statistical analysis of the data. The effectiveness of the EO (0.3 %) and of its hydrolate (14.7 %) was correlated with the main chemical components (i.e. thymol, *p*-cymene). From the results obtained in this first trial *in planta*, the possibility of using in the field the *M. didyma* EO to control the bacterial canker of kiwifruit is discussed.

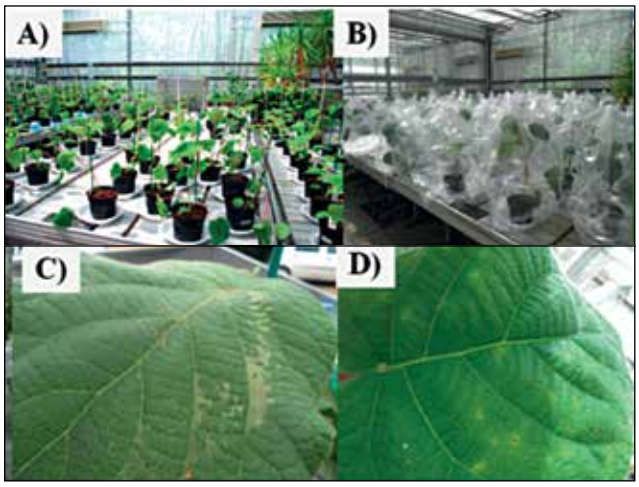

Fig 6. Micropropagated plants of A. deliciosa cv Tomuri used in this study before (A) and after (B) the four treatments; C) Phytoxicity caused in some plants by EO (0,3%); D) Leaf spots induced by Psa 8 days after inoculation.

Fig. 7. M. didyma.

ANALYSIS OF THE VOLATILE COMPOUNDS OF AERIAL PARTS AND ESSENTIAL OIL FROM *THYMUS SERPYLLUM* L. CULTIVATED IN NORTH EAST ITALY BY HS-SPME/GC-MS AND EVALUATION OF THEIR FLAVOURING EFFECT ON RICOTTA CHEESE

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Headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography and mass-spectrometry (GC-MS) was applied for the analysis of flavour profiles of *Thymus serpyllum* L. aerial parts, the corresponding essential oil (EO) and ricotta cheese flavoured with the EO. By direct GC/MS analysis, the EO composition was determined. The flavouring effect of different amounts of EO (0.2, 0.3 and 0.4 g) added to 100 g of ricotta cheese was studied. Significant differences ($p \leq 0.05$) were found for monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons only when 0.4 g of EO was added. The hydrocarbons monoterpene and sesquiterpene were retained by ricotta, whilst oxygenated monoterpenes were released.

THE EFFECT OF ESSENTIAL OILS WHEN INTEGRATED IN CHICKEN DIET

Vasil Pirgozliev

The National Institute of Poultry Husbandry, Harper Adams University, Shropshire, England

The aim of the present work was to investigate the effect of a mixture of essential oils (EO) including carvacrol, cinnamaldehyde and capsicum oleoresin on growth performance, dietary metabolisable energy (ME), nutrient digestibility, hepatic antioxidant content and the relative expression of some genes when fed to broiler chickens. Feeding the EO mixture improved growth, feed efficiency and ME intake ($P<0.05$). It also improved antioxidative status of birds, increasing hepatic vitamin E and coenzyme Q_{10} contents ($P<0.05$), and also changed some cytokine gene expression in caecal tonsils showing an immune modulating effect. The results suggest that feeding EO under commercial conditions may have the potential to improve the birds health status and reduce the impact of disease challenges.

SENSORY EXPERIENCES WITH ESSENTIAL OILS AND FOOD

Roberta Deiana

Author, food writer and food stylist – Milano, Italy.

The act of eating engages all five senses. However, the gastronomic experience can become richer and more fulfilling with the help of essential oils, and particularly for taste, smell and sight.
As for hearing and touch, dishes can become more stimulating by simply juxtaposing ingredients with different textures.
Sight can be enhanced by using decorations referring directly to the dominating essential oil in the dish, be it in colours or shapes.
As for taste and smell, the choice is extensive: not only essential oils can help creating infinite combinations of ingredients and flavours (since they are easier to combine than barks or zests) but they enhance the original spice or fresh herb: if used in synergy, they convey a surprising sense of tridimensional taste.

Coltivazione di lavanda nel Giardino delle Erbe di Casola Valsenio (RA)

ESSENTIAL OILS IN VETERINARY MEDICINE: TESTS OF SOME ESSENTIAL OILS AND OF A MIXTURE TOWARDS STRAINS OF MULTIDRUG-RESISTANT ORGANISMS OF *ESCHERICHIA COLI*

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Using the technique of microdilution in microtiter plates with 96 wells, this work evaluated the minimum inhibitory concentration (MIC) of some essential oils (Coridotymus capitatus, Melaleuca leucadendron, Eucalyptus globulus) and of a mixture of essential oils towards a number of strains of Escherichia coli. The test was made on multidrug-resistant organisms selected from pig and poultry species (chicken and turkey) in breeding farms. This work also evaluated the MIC towards strains of Escherichia coli selected from wild birds. The aim of this work was to study the possible synergistic effects between the individual essential oils and the mixture of essential oils, and between this mixture and the antibiotic amoxicillin.

ESSENTIAL OILS FOR FOOD PRESERVATION

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The application of essential oils (EOs) in food products has been demonstrated to be effective in both controlling the growth of pathogens and extending food shelf-life. Although they are active at low concentrations *in vitro*, higher quantities (≥ 1.0%) are required for food preservation, with possible detrimental effects on the sensory characteristics. Moreover, in complex matrices EOs show prompt antimicrobial activity, which is generally unstable over time. Therefore, researchers are exploring new strategies based on the *hurdle technology* approach, where EOs are either combined with other antimicrobials or encapsulated to improve their stability and activity. In the light of these considerations, EOs offer good perspectives of application for food preservation, but further research is needed to optimize treatments.

MENTHA X PIPERITA (HUDS) (*LAMIACEAE*) ESSENTIAL OIL OF PANCALIERI (TURIN, ITALY): PRELIMINARY EVALUATION OF THE ANTIFUNGAL ACTIVITY AND SYNERGISTIC INTERACTION WITH ANTIFUNGAL DRUGS

Daniela Scalas, Janira Roana, Narcisa Mandras, Giuliana Banche, Valeria Allizond, Anna Maria Cuffini, Vivian Tullio

Department of Public Health and Paediatrics, University of Torino, Microbiology Section, Torino, Italy

Mentha x piperita L. has been used in folk medicine since antiquity. Recent studies have shown that its essential oil (EO) has antimicrobial properties. We evaluated the antifungal activity of peppermint Black Mitcham of Pancalieri EO against 16 *Candida spp.,* 15 non-*Candida spp.*, and 5 dermatophyte strains (*Microsporum canis*, *M.gypseum, Trichophyton mentagrophytes*). Moreover, we evaluated the synergism of peppermint EO with azole drugs (itraconazole and/or ketoconazole) through the checkerboard method. Our preliminary study suggests that peppermint of Pancalieri EO exerts a fungicidal activity against yeast cells, and a fungistatic activity against dermatophytes. Interactions of peppermint EO with azole drugs *vs. Cryptococcus neoformans* and *T.mentagrophytes* indicate mainly synergistic profiles.

Fig. 8. Mentha piperita.

ANTIBACTERIAL, ANTIFUNGAL AND ANTIVIRAL ACTIVITIES OF *CORIDOTHYMUS CAPITATUS* (L.) RCHB.F. HYDROLATE FROM SCOGLITTI (SICILY)

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The aim of this study was to evaluate the susceptibility of bacteria, yeasts and viruses to the hydrolate of *Coridothymus capitatus* (L.) Rchb.f. that contained 0.6% essential oil. Characterization and toxicity were evaluated. The antimicrobial screening was tested against staphylococci (including methicillin-resistant strains), *Listeria monocytogenes* spp., *Pseudomonas aeruginosa*, *Candida* spp. and *Herpes Simplex* Virus type 1 (HSV-1) and type 2. Studies on the effects of hydrolate in combination with antimicrobial drugs were performed. Carvacrol was the main compound detected. The hydrolate was non-toxic, and showed the highest antimicrobial activity against *S. aureus*, *C. glabrata* and HSV-2. A synergistic effect was obtained between hydrolate and itraconazole (ITZ) against ITZ-resistant *C. krusei*.

NANOMEDICINE AND ESSENTIAL OILS

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Department of Technology and Health, Istituto Superiore di Sanità, Roma, Italy

Nanomedicine is the application of materials, devices and processes from nanotechnology to develop nano-sized tools for the diagnosis, prevention and treatment of diseases. Nanocarriers can improve the stability of Essential Oils (EOs) against degradation and the key issue is to achieve their desired concentration in tumour sites.
Various drug delivery systems such as nano/microparticles and liposomes have been demonstrated to significantly enhance the preventive/therapeutic efficacy of many drugs by increasing their bioavailability and targetability. Recently, several studies have demonstrated that the incorporation of EOs into nano-microparticles is able to increase their stability.

FIRST PRELIMINARY *IN VIVO* DETERMINATION OF THE EFFICACY AND SAFETY PROFILE OF A COMBINATION THERAPY BASED ON PROBIOTICS AND TEA TREE OIL IN VAGINAL MYCOSIS

Giuseppe Fracchiolla[1], Maura Di Vito[2,3], Annunziata Tamburro[3], Milva Ballardini[3], Silvana Maiorano[3], Giuseppe Testa[3], Francesco Padula[4], Luigi Agatensi[5], Francesca Romana Giorlandino[4], Paola Mattarelli[6], Monica Modesto[6], Giuseppe Gerardo Carbonara[1], Rossana Bellone De Grecis[1], Filomena Corbo[1], Francesca Mondello[2]

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Tea tree oil (TTO), an essential oil extracted by steam distillation from the leaves of the Melaleuca Alternifolia tree, has been known for many years for its antiseptic properties. Currently, the TTO is used in vaginal suppositories (VS) for the vaginal candidiasis treatment. TTO-VS exhibits an *in vitro* fungicidal activity towards Candida spp., slightly affecting some vaginal probiotics isolated from patients with vaginitis. However, preclinical and clinical studies are still missing, as well as studies on the efficacy and safety profile, especially in the complex vaginal microbial environment. Given these interesting results, we are pursuing *in vivo* studies to evaluate the efficacy and safety profile of a combination therapy with probiotics and TTO-VS on female volunteers with vaginitis.

COMPARATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF SOME ESSENTIAL OILS AGAINST MULTI-RESISTANT BACTERIA OF CLINICAL ORIGIN

Maura Di Vito, Monica Monaco, Maria Giufrè, Antonietta Girolamo, Francesca Mondello

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The indiscriminate use of antibiotics has led to the emergence of drug-microbial resistance in addition to an increase in allergies and unexpected adverse drug reactions. In particular, the fight against nosocomial infections involving microorganisms resistant to most or all available antibiotics, has become a major concern worldwide. New safe and effective antimicrobial alternatives are urgent. Among the most potential candidates in the prevention and treatment of various infectious diseases, there are mainly products of natural origin, such as essential oils (EOs). From our *in vitro* data, the EOs of the fruits of *Trachyspermum ammi* L., *Melaleuca alternifolia* and its main component terpinen-4-ol are innovative and promising microbicides against emerging threats to public health, such as *Staphylococcus aureus* (MRSA), KCP *Klebsiella pneumoniae*, ESBL *Escherichia coli*.

ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS OF LAVENDER AND ITS HYBRIDS AGAINST DIFFERENT FUNGI OF ENVIRONMENTAL ISOLATION

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Fig. 9. Lavandula hybrida "Grosso"

Fig. 10. Lavandula hybrida "Abrialis"

Essential oils are well known for their antibacterial, antimycotic and insecticidal effects. The Lavandula genus, which includes lavender and lavandin, is cultivated worldwide for its essential oils. The chemical composition of the essential oils is characterized by the presence of terpenes (e.g. linalool and linalyl acetate) and terpenoids (e.g. 1,8-cineole), which are mainly responsible for their characteristic flavour and their biological and therapeutic properties. Five essential oils of lavender and lavandin were tested for their antifungal activity using a microtiter assay: the minimal lethal concentration (MLC) was determined against different fungi. *Penicillium glabrum*, *Fusarium* spp. and *Ceriporia* spp. were the most sensitive fungi, followed by *Aspergillus* species.

QUALI-QUANTITATIVE CHARACTERIZATION OF ESSENTIAL OILS IN RELATION TO DIFFERENT EXTRACTIVE PARAMETERS

Marco Valussi

European Herbal & Traditional Medicine Practitioner Association (EHTPA) Verona, Italy

The last 10 years have witnessed a great increase of interest in the chemical characterization of EOs. The appropriate choice of genetic material, the matching of plants with the correct growing environment and the use of correct analytical techniques have all been discussed in the lay and professional literature. Less attention has been paid to the parameters of distillation. However, the type of distillation, the choice of pressure, the steam flow intensity, the wetness of materials, the grade of comminution, etc., can have a profound influence both on the chemical quality and on the quantity of Eos, and are capable of negating the choice of genetic material. In this paper, I offer a review of the existing published literature as well as present the result of a study on the effects of distillation parameters on *Pinus mugo* EO.

ESSENTIAL OILS AND ANTIMICROBIAL AGENTS: IS THEIR ACTIVITY COMPARABLE?

Vivian Tullio, Narcisa Mandras, Janira Roana, Daniela Scalas, Valeria Allizond, Elisa Marra, Giuliana Banche, Annamaria Cuffini

Department of Public Health and Paediatrics, Microbiology Section, University of Torino, Italy

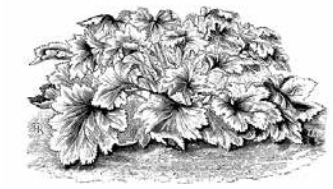
The growing threat of antimicrobial drug resistance has stimulated the search for new therapeutic alternatives, including essential oils (EOs) that are now recognized for their potential antimicrobial role against microorganisms. Since clinical experience has showed that the efficacy of antimicrobial drugs depends both on their direct effect on a given microorganism and on the activity of the host immune system, we have evaluated the activity of several EOs against yeasts and filamentous fungi and the influence of thyme red oil on intracellular killing by PMNs against *Candida* spp., in comparison with azoles and echinocandins. Data have showed a good activity of EOs and suggested a positive interaction of thyme red oil with PMNs in eradicating intracellular yeasts, as observed with antimicrobial drugs.

AROMATHERAPY AND PEDIATRIC INFECTIONS

Dominique Baudoux

Director of the Research Laboratory - Pranarom – Belgium

Eating organic food, breathing healthy air, healing naturally or, if you prefer, fleeing from this synthetic chemistry that invades and poisons us on a daily basis in insidious ways. These are the demands of new parents facing the medical world to treat their babies. It is obvious to say that 50% of the symptoms in paediatrics find their origin in a viral cause: cough, flu, colds, otitis, bronchitis... You cannot give them antibiotics since they have never destroyed the smallest virus. And for the infant's eczema, do you really think that cortisone is a lasting solution? And what to do to relieve the pain of teething...
Fortunately , the pharmacological rediscovery of the oldest therapeutics in the world brings an innovation confirmed on many occasions by more and more numerous clinical studies. Here are a few simple protocols to open the spirit... at the very least, and even more to see them applied to our dear toddlers.



Heuchera glabra

A SINGLE-CENTRE PROSPECTIVE RANDOMIZED CLINICAL TRIAL ON THE TREATMENT OF TRANS-SPHINCTERIC IDIOPATHIC ANAL FISTULA WITH *LAVANDULA ANGUSTIFOLIA* ESSENTIAL OIL: PRELIMINARY RESULTS

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Purpose: The treatment of complex anal fistula often hesitates in a perianal wound with slow healing.
Patients and methods: From April to August 2015, 25 patients underwent LIFT surgical technique and were divided in two groups. Postoperatively, group A patients were treated with E.O. of *Lavandula angustifolia* 8% in almond oil carrier. Group B patients were medicated with Dakin solution.
Results: All patients (group A and B) healed. No recurrence was observed. The mean healing-time in group A patients was 20.5 days (range 14-28) vs 34.3 days (range 21-42) of group B (p=.0003).
Conclusion: Preliminary results show a significant reduction in the healing-time of perianal wounds of group A patients. There are no data yet about the rate of recurrence because of short-time follow up.

Fig. 11. Lavandula vera.

Una veduta del Giardino delle Erbe di Casola Valsenio (RA)

SYNERGISTIC ACTIVITY OF ESSENTIAL OILS

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Because of the emergence of multi-drug resistant microorganisms, alternatives to conventional antimicrobial therapy are needed. This can be achieved by the use of combinations of existing agents or the development of new, safer and effective agents primarily from plant sources which can exhibit synergy with drugs. In particular, it has been demonstrated that the combination of *Mentha suaveolens* essential oil (EOMS) with different drugs is synergic in various microorganisms. Indeed, the combination of EOMS plus fluconazole in *C. albicans* is synergic with FIC index of 0.375, or plus gentamicin in *Klebesiella pneumoniae* with FIC index 0.5. The same essential oil plus erythromycin is able to inhibit *Clamidiae trachomathis* replication or in the combination with acyclovir HSV-1 replication.

Bulbocodium trigynum

ESSENTIAL OIL CARRIERS EVALUATION

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The study carried out has the aim to investigate which are the ideal means to convey essential oils through the skin and for oral ingestion. Initially, a series of systematic tests was carried out to evaluate the solubility of essential oils in the several different vehicles taken (hydro-alcoholic lotion, o/w emulsion, gel, oil) and to monitor the organoleptic characteristics, the stability and the rheology of the samples obtained.

The choice of the essential oils used for the tests was carried out on the basis of data relating to sales volumes, which in part reflect the list of oils of greater interest for their properties, and according to the chemical structure of the essential oils (hydrocarbons, alcohols, aldehydes , ketones, esters, ethers).

This aspect is very important to get information relating to the correlation between solubility and composition and therefore to predict how other essential oils could react with the analysed vehicles.

According to the data obtained in this preliminary work, we chose the formulations that proved to be the most stable, with three different concentrations of essential oil. In further studies, we will measure transdermal permeation for these

formulations.
The permeability will be assessed on creams and ointments containing any of the following essential oils: sweet orange, lavender, tea tree, ginger.

TOPICAL ESSENTIAL OIL OF *LAVANDULA ANGUSTIFOLIA* IN A HIPPOCRATIC MELANCHOLIC CONSTITUTION/CHINESE METAL CONSTITUTION PAEDIATRIC SUBJECT

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Essential oils have been linked to the Essence both in Hippocratic medicine and in the Chinese one. Hippocratic medicine is based on the Elements/Essence of Empedocles. Chinese medicine knows three treasures: Jing/Essence, Qi and Shen/Spirit.
Following the teaching of the 4 Hippocratic humours, you can identify 4 constitutions: phlegmatic, melancholic, choleric, sanguine. The Chinese doctrine of the 5 transformations identifies 5 constitutions: wood, fire, earth, metal, water. Each constitution, Essence-related, can be paired with an Essential Oil.
We report a case of excessive shyness treated with the Essential Oil of *Lavandula angustifolia* by massage on GV12.

A BRIEF INTRODUCTION TO AROMATHERAPY OF THE TERRAIN ACCORDING TO ENDOBIOGENY

Jean Claude Lapraz MD[1,2], Kamyar M. Hedayat, MD[1,3]

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Aromatherapy offers many advantages in the care of patients but tends to be used symptomatically and as a substitute for medications. Clinical aromatherapy is the rational and systematic application of essential oils to the global terrain of the patient. It offers a more physiologic way of selecting and applying essential oils in clinical practice. We present the theory of Endobiogeny as an example of approach to integrative physiology and clinical aromatherapy. The efficacy of endobiogeny has been demonstrated by using the application of *Salvia officinalis* in a post-menopausal woman. In this example, six months of treatment using low-dose oral and vaginal *Salvia officinalis* allowed for resolution of symptoms and correction of the terrain.

TOPICAL USE OF ESSENTIAL OILS ON CONSTITUTIONAL BASIS ACCORDING TO CHINESE MEDICINE

Francesco Deodato

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Knowing the constitutional ground on which a disease develops may allow to understand its evolution and the base that has caused it to a good approximation. Traditional medicines have the peculiarity of being born when everything had to be classified clinically and quickly, in absence of equipment and laboratories. Chinese medicine, to which this work refers, dates back to thousands of years ago and represents a very interesting patrimony for diagnostic and therapeutic integration. Essential oils, which are considered the purest and more concentrated essence of a plant by Chinese medicine, can produce very important changes in individual health, if properly selected and applied with a massage on specific acupuncture points.

Olio essenziale di lavandino

COSMETIC AND PHARMACEUTICAL PREPARATIONS

Franco Bettiol

Kos s.r.l. and Galeno s.r.l. Technical Manager. Comeana, Carmignano (PO), Italy

1. Essential Oils in preparations for cutaneous application: Some examples of cosmetic and pharmaceutical preparations (main Essential Oil in round brackets).
2. Liquids: Hydroalcoholic L.: Antifungal Lotion (Myrrh), Anti-pediculosis Lotion (Ylang-ylang), Cold Bandages (Eucalypt). Oily L.: Anti-pediculosis Lotion (Tea-tree), Lymph-drainant Massage Oil (Cypress), Cellulite Massage Oil (Pepper, Juniper).
3. Cleanser: Intimate wash (Lavender).
4.Gels: Hydrogels: Chilblains gel (Rosemary). Lipogels: Wearing lipogel (Rosemary, Oregano).
5. Creams**:** Protective Cream (Tea-Tree)
6. Ointments: Antirheumatic Ointment (Juniper), Anti-Fissure Ointment (Lavender)

EMOTIONAL AROMATHERAPY AND COSMETICS

Elena Cobez

Aromatherapy and Botanical Perfumery Trainer – Milano, Italy

The holistic wellbeing of each of us is based on the harmonical relationship with our body, our emotions, our mind and our personal history. This harmony is more an horizon than a reality for the majority of people. Aromatherapy is one of the most suitable instruments for the emotional support, as an everyday element that may partner us in our different existential phases. From this point of view, using aromatherapy cosmetics is an instrument of a multilevel wellbeing, energizing the skin and the psyche. The skin is not only a physical surface, but also a symbolic one, where we experience biochemical exchanges and symbolical exchanges.

INNOVATIVE METHODOLOGIES IN NANOTECHNOLOGY FOR THE PREVENTION OF BACTERIAL DISEASES TOWARDS THE DEVELOPMENT OF NEW MATERIALS FOR SANITIZING PROCESSES SIMPLY BY USING ESSENTIAL OILS

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In the last decades, research has been devoted to the development of new products for enhancing the quality of human life. The antimicrobial agents, generally used to avoid the production of unpleasant odours or the increase of potential health risks, are some of these products. Therefore, various antibacterial finishing and disinfection techniques are being developed to be applied, from hospital environment (medical clothes, protective garments, etc.) to everyday household and clothing. Almost every class of chemical products, ranging from the very simple substances such as halogen ions to very complex compounds, has been tested to confer the antibacterial activity to one material. But the use of many of these antimicrobial agents without a tight control over their bioactivity has been stopped because of their possible undesired harmful or toxic effects. Moreover, one of the most widespread problems related to the indiscriminate use of antibiotics consists in the onset of resistance mechanisms (AMR) that make these drugs inactive.

AMR is the resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it. Resistant microorganisms are able to withstand attacks by antimicrobial drugs, so that standard treatments become ineffective and infections persist, increasing the risk of spread to others. The other major factor in the growth of antibiotic resistance is the spread of resistant strains of bacteria from person to person, or from the non-human sources in the environment, including food. In fact, antibiotics are also commonly used in food animals to prevent, control and treat diseases, as well as to promote the growth of food-producing animals.

Recently, the market for natural products from plant extracts, dry extracts and essential oils, and their use as "Green Advanced Materials", is undergoing a rapid expansion following the now recognized antimicrobial action spectra extremely large in these substances. The properties of the natural products resulting from the presence of bioactive molecules are capable of performing various pharmacological activities, antimicrobial, antioxidant and flavouring.

Recent guidance from the U.S. Food and Drug Administration (FDA), accepted also by European Health Agencies, describes a four core action pathway for Fighting back against Antibiotic Resistance.

1 Preventing infections and preventing the spread of resistance;
2 Tracking resistant bacteria;
3 Improving the use of today's antibiotics;
4 Developing new drugs and diagnostic tests.

Our research activity fits exactly in this last point with the final aim of developing new materials for sanitizing processes of environments and work ambient simply by using essential oils.

The chemical composition of EOs was determined by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS). The analysis was carried out on HP GC/MS 6890-5973 MSD spectrometer, i.e. 70 eV, equipped with HP ChemStation and MS NIST library. The similarity index of the spectra (SI) and the linear retention index (LRI) experimentally determined were compared with those reported by the MS libraries used.

We started with a chemical identity screening of new potential antimicrobics synthesized by us and with a selection of essential oils which were chemically characterized and standardized.

We selected two synthetic compounds that were related, in terms of space and shape, to the quinolones scaffold and in particular to Levofloxacin, used as standard compound in our biological assay. The shape analysis of the new compounds was carried out using ROCS software.

The antimicrobial activity of new molecules, essential oils and their synergic combination on a wide series of bacterial strains as planktonic cells and bacterial biofilms was investigated.

The MIC values of the two most active derivatives SRA71 and FS11 were compared to Levofloxacin MIC value. Certainly, Levofloxacin was about one to two orders more active than the tested compounds. However, the antimicrobial spectrum of FS11 was similar to that of the standard compound. Given these interesting results, we carried out an in-depth molecular docking study based on the available crystallographic data of Levofloxacin crystallized into the SA topoisomerase active site. As observed, the interaction of Levofloxacin occurs with the four amino acid residues of enzyme binding pocket. Even the compounds SRA71 and FS11 fit into the enzyme binding pocket and are able to interact with arg144, but fail to form a dense network of hydrogen bonds with other amino acid residues of the enzyme pocket.

In addition, the antimicrobial activity of the synergies created by the EO in combined use with zinc oxide or other nano- or micro-phases, having known antibacterial properties, will be presented and discussed.

In collaboration with the research group of Prof Antonio Valentini, a method of deposition of essential oils on inert supports exploiting the adhesion properties of the ZnO microparticles was developed. With the aim to prepare novel modified-release devices, a method of spray deposition using a PC-controlled airbrush was employed. However, different Petri plates were prepared, where the essential oil ZnO and the Oil-ZnO dispersions were deposited uniformly as evidenced by the amount of deposited material.

To test the antibacterial effect of the devices made, we

developed an original experimental protocol that could simulate bacterial contamination of surfaces or environments. This protocol foresees the monitoring of bacterial growth in time, after inoculum of 100 thousand cells, and could be considered a stress test for the plates since it is a high degree of contamination compared to a normal situation in disinfected environments.
3 Petri plates for each sample of ZnO, essential oil and ZnO/EO Association were preliminarily treated with UV radiation to remove any bacterial contamination of the deposition steps. Subsequently, all the plates were inoculated with MRSA and treated with a culture medium favourable to the growth of bacteria and the formation of biofilm. After 24 hours of incubation (time 0), all the samples were monitored by taking an aliquot of culture medium and by performing the bacterial count. As can be observed between day 1 and 4 of inoculation exposure, the control plates grew up to 10/8 while bacterial growth was not observed in the deposited plates. From day 4 to 21, the plates treated began providing a bacterial growth up to two orders of magnitude lower than the control. This bacteriostatic effect was maintained up to day 30. Subsequently, the samples showed a growth comparable to the control.

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CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *PLUMBAGO EUROPEA* L. ROOTS (PLUMBAGINACEAE) AND EVALUATION OF THE CYTOTOXIC ACTIVITY OF ITS MAIN COMPONENT, PLUMBAGIN

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Introduction

The genus *Plumbago* (Fam. Plumbaginaceae) comprises about 12 species which widely occur in Europe, especially in the Mediterranean region, Spain, France, Northern and Southern Italy, North Africa and Southwest Asia. *Plumbago zeylanica* L. is the species that has been studied the most, whose root extract is well known for its antiviral, anti-microbial, nematocidal, anti-inflammatory and cytotoxic activities [1]. Other species belonging to the genus *Plumbago* have been investigated less and few references about it are reported in the literature. One of these is *Plumbago europea* L., also named "caprinella europea" or "piombaggine", which wildly grows along the coast of the largest Italian islands, Sardinia and Sicily, Liguria and Southern Italy [2].

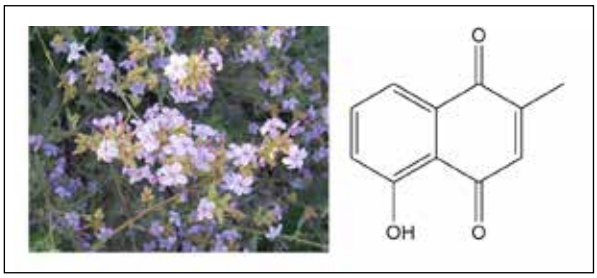

Figure 1. Plumbago europea L. (at http://www.funghiitaliani.it/index.php?showtopic=31991) and structure of plumbagin

As we characterized extensively the chemical composition of the essential oil of plants belonging to the spontaneous Italian flora, in the present communication we wish to report the chemical composition of the essential oil obtained from fresh roots of *P. europea* collected in Apulia. We also report the cytotoxic activity of the main component isolated from this essential oil. In particular, we studied the *in vitro* IC_{50} growth inhibitory concentration on a panel of six human cancer cell lines exhibiting different levels of resistance to pro-apoptotic stimuli by means of the MTT colorimetric assay and quantitative videomicroscopy.

Materials and Methods

The essential oil was obtained from fresh roots of *P. europea* collected in Apulia, in particular in Salento peninsula, using a Clevenger-type hydrodistillation apparatus, and then analysed by gas-chromatography coupled to mass spectrometry (GC/MS) as reported in literature [3]. The E.O. was then purified by preparative silica column chromatography using dichloromethane as the mobile phase. The main compound isolated was assayed for its cytotoxic activity by MTT colorimetric assay and quantitative videomicroscopy [4].

Results and Discussion

The sample oil, which was viscous and yellow in colour, was obtained in a yield of 0.16% p/p. The literature reveals only one reference on the E.O. from roots of *P. europea* [5]. Navaei et al. collected the roots of the wild plant under study in Teheran, Iran, where fifteen components were identified. The major constituents were plumbagin (69.1%), 1-octen-3-yl acetate (9%) and limonene (5.7%). The E.O. from roots of Italian wild *P. europea* was shown to be richer in plumbagin (84.7%) and with less amount of 1-octen-3-yl acetate (4.6%), limonene (4.2%) and other components like monoterpenes (linalool, thymol and carvacrol) and hydrocarbon sesquiterpenes (germacrene and

β-bisabolene). Among all components, plumbagin (Figure 1) was extensively studied and was found to exert anticancer properties and cytotoxicity activities, modulating cell proliferation. For this reason, we decided to isolate the active 1.4-naphtoquinone and then study its *in vitro* IC_{50} growth inhibitory concentration on a panel of human cancer cell lines (Hs683 glioblastoma cells, A549 non-small-cell lung cancer cell line, MCF7 breast cancer cell line, SKMEL-28 melanoma and U373 glioblastoma cells) and mouse B16F10 (melanoma) exhibiting different levels of resistance to pro-apoptotic stimuli by means of the MTT colorimetric assay. Plumbagin showed to exert a valuable growth inhibitory activity on all cell lines mentioned with an IC_{50} mean value of 4±1 μM, from 1μM on B16F10 to 5μM on SKMEL-28 and U373. Then, we submitted the human U373 GBM cell line to quantitative videomicroscopy analyses at its IC_{50} value (5μM) in order to determine whether the compounds studied induced cytotoxic or cytostatic effects. Data revealed that all compounds completely halted cell growth and morphological analysis revealed that a cytotoxic rather than a cytostatic effect induced numerous cell deaths.

Conclusion

In conclusion, in this study we characterized for the first time the chemical composition of the essential oil of *Plumbago europea* L. collected in Italy. In particular, the essential oil obtained from the wild plant from the coast of Salento peninsula showed a high amount of the bioactive compound, plumbagin. Plumbagin was shown to inhibit selected pro-apoptotic resistant cancer cells growth *in vitro*.

Acknowledgments

The authors wish to thank the University "Gabriele D'Annunzio" of Chieti for its financial contribution and Prof Robert Kiss and Prof Veronique Mathieu from the Université Libre of Bruxelles, who allowed the author of this communication to perform the toxicological assays.

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THE ESSENTIAL OILS AS NEW RESOURCES OF HUMAN CARBONIC ANHYDRASE INHIBITORS IDENTIFIED BY *IN SILICO* TECHNIQUES

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Theoretical studies and *in silico* methods, by means of high performing computers, today represent new innovative approaches to the drug discovery process. In fact, excluding the clinical trials, chemoinformatics and bioinformatics are playing important roles in every stage of drug discovery pipeline (Image 1) [1]. The Essential Oil University (EOU) website is an example of free chemoinformatics database, providing accurate information about the world of essential oils. In this large resource, a huge amount of analytical reference data are collected for research and quality control education purposes [2]. In particular, for our study, the 3D chemical structures of 2690 compounds, contained in the EOU website, have been downloaded in order to perform a structure-based virtual screening (SBVS) for identifying new hits active against several medicinal chemistry targets.

Among these, there are Human Carbonic Anhydrases (hCAs), zinc-containing metalloenzymes that catalyse the reversible hydration of carbon dioxide according to the following reaction:

$$CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$$

Sixteen hCA isozymes have been described up to now in mammals [3] and some of these (e.g. hCA II, VA, IX, XII) constitute targets for the development of antiglaucoma, diuretic, antiobesity, anticonvulsant or anticancer drugs [4]. The X-ray crystal structures of the targets selected to perform docking simulations are deposited into the bioinformatics Protein Data Bank (PDB) [5], considering essential oils as ligand-library, and already used for a previous SBVS [6,7]. Ten *in silico* identified hits out of more than 2500 entries, i.e. theoretically the most promising molecules, have been submitted to *in vitro* assays in order to focus directly on

Image 1: Flowchart of in silico methods used in every stage of the drug discovery process.

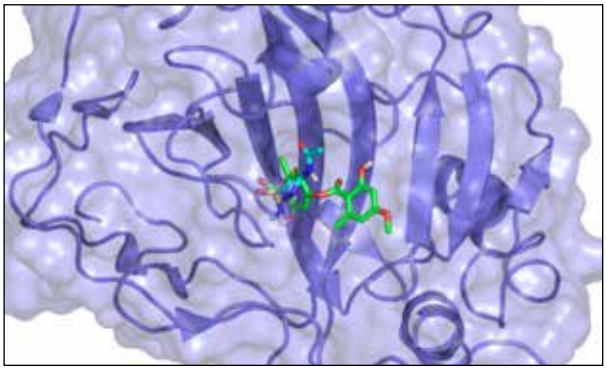

Image 2: Molecular recognition of one hit compound (carbon-green sticks) and superimposed to the reference co-crystallized inhibitor acetazolamide (carbon-cyan sticks).

novel hCA interfering compounds (Image 2).
Preliminary results confirm the presence of hCA inhibitors among the 10 selected compounds, mostly active in the order of low micromolar concentrations. Some of them have resulted also as selective inhibitors against specific hCA isoforms, with potential novel applications of essential oils containing them in consistent amount in Carbonic Anhydrases related diseases.
This work confirms the important role of SBVS techniques to speed up the identification of bioactive compounds also in the field of essential oils, reducing time and costs of research procedures.

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ANTITUMORAL ACTIVITIES OF THREE ESSENTIAL OILS

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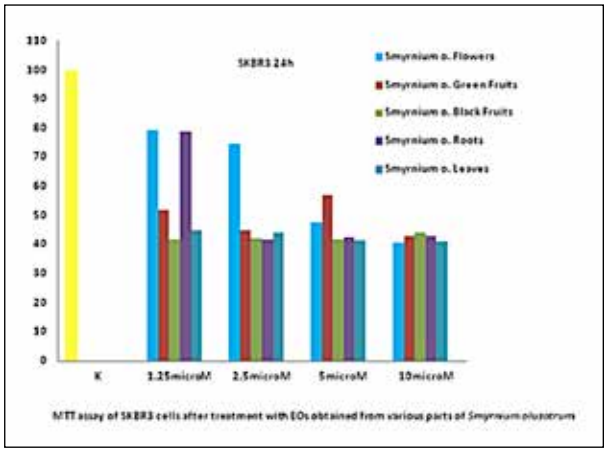
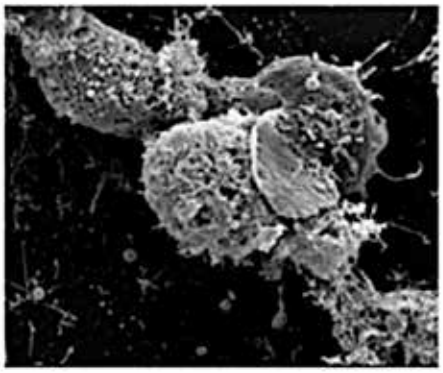
Natural compounds have been used extensively in medicinal chemistry. They contain several bioactive molecules that synergistically provide therapeutic efficacy. Several drugs derived from natural products have already received clinical approval and many are currently enrolled in clinical trials. Essential oils (EOs) are products obtained from vegetable raw materials, either by distillation with water or steam or by dry distillation (*Celia, 2013*). They are complex, multi-component systems composed of volatile small molecular weight components, mainly terpenes and non-terpene components. EOs have been used for a long time by various traditional medicine systems as antiseptic agents (*Rios and Recio*, 2005). Nowadays, scientific evidence is available showing that specific EOs have antimicrobial, antimycotic, antiviral, antioxidant, immunomodulatory and anticancer properties (*Edris, 2007; Lang and Buchbauer, 2012*). Some of these characteristics are related to their functions in the plants. Actually, it has been recognized that various EOs components act as multi-target molecules. With the aim of developing novel anticancer drugs characterized by selective targeting and low toxicity for normal dividing cells, we have devoted our attention to various EOs that have showed no toxicity to normal cells, and we have evaluated their efficacy against human cancer cells. In particular, we have studied cytotoxicity of three EOs (*Smyrnium olusatrum* L., *Mentha suaveolens* and *Vepris macrophylla*). *S.-olusatrum* L., belonging to the family of Apiaceae, known in Italian as 'Macerone' or 'Sedano selvatico', is a stout, glabrous, celery-scented biennial herb, up to 60 inches tall. *S. olusatrum* occurs in central and southern Italy and in the islands while, in the northern part, is widespread, mainly in Emilia-Romagna (*Maggi, 2012*). Among them, one of the main components is isofuranodiene, reported to possess significant anticancer and anti-angiogenic activity (*Zhong, 2011*). *M. suaveolens* essential oil is obtained from wild-type plants grown in Tarquinia forests, located around 60 miles from Rome. Analysis has revealed that piperitenone oxide constitutes 90% of oil (*Pietrella, 2011*). The last one, *V. macrophylla*, is an evergreen tree occurring in sub-humid forest of Madagascar and traditionally used in tea. The major compounds are citral, mixture of neral and geranial, citronellol and myrcene. Cytotoxicity of these EOs has been studied on human breast adenocarcinoma cell line SKBR3. This evaluation of cellular metabolism has been analysed by MTT as-

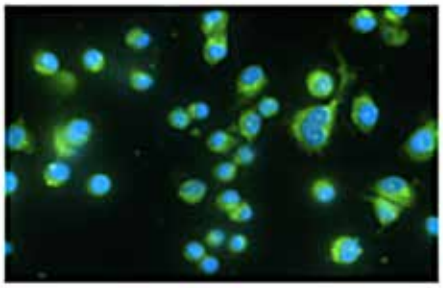
say at different concentrations of EOs and at different times of incubation (24, 48 and 72 h). Moreover, morphological and ultrastructural studies have been performed to understand the EOs antitumoral action against human tumoral cells. Some results are shown in Fig.1.



MTT assay of SKBR3 cells after treatment with EOs obtained from various parts of *Smyrnium olusatrum*



Scanning electron microscopy observations of SKBR3 cells incubated with *Mentha suaveolens* EO for 24 h at 0.03 mg/ml.



Fluorescence microscopy observations of SKBR3 cells after treatment with *Vepris macrophylla* EO for 24h. Green indicates actin filaments while blue indicates nuclei stained with Hoechst 33342.

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LAMIACEAE ESSENTIAL OILS: PHYTOCHEMICAL CHARACTERIZATION AND *IN VITRO* BIOLOGICAL ACTIVITY ON *STREPTOCOCCUS AGALACTIAE*

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Essential oils (EOs) are secondary metabolites produced by aromatic plants special glands for defensive purposes and they are among the most important phytocomplexes with a high antimicrobial activity. EOs are obtained in most cases by means of steam distillation [1,2]. EOs therapeutic properties have been known since ancient times and are still subjects of particular interest [1,2]. The Mediterranean area is famous for being rich in aromatic plants, endemic sources of peculiar EOs. Plants belonging to the *Lamiaceae* family are cultivated

in this area and they are of considerable interest due to their biodiversity and to the different monoterpenes produced by several genera and species. In the last decades, researchers have been particularly interested in these plant compounds in order to investigate both their chemical composition and biological properties. Nevertheless, further investigations are still needed to confirm their real activities on the basis of rational EO composition-antimicrobial activity studies on many microorganisms [1,2]. In this study, attention was focused on the bacterium *Streptococcus agalactiae* (GBS), the agent of infections in adults with diabetes or chronic illnesses and also a major contributor to severe infections such as sepsis, meningitis, pneumonia in newborns. Indeed, for this reason, pregnant women are closely monitored in the months before childbirth: if *S. agalactiae* colonized the mother's genital tract, there would be a high probability of vertical transmission in childbirth [3]. For this study, four EOs belonging to the *Lamiaceae* family were selected, kindly provided by *Alchimia Natura* s.r.l. Laboratory. These EOs were obtained from plants grown in accordance with the principles of natural, organic and biodynamic agriculture in the territory of the Modena Apennines, in particular: *Lavandula x intermedia* Emeric ex Loisel., *Origanum vulgare* L., *Satureja montana* L. and *Thymus vulgaris* L.. All EOs were subjected to qualitative and semi-quantitative analysis in order to obtain their phytochemical characterization. The gas chromatographic analysis was performed by GC-MS and GC-FID respectively. An HP-5 gas chromatographic column was chosen and a unique thermic ramp was optimized for all samples analysed. The qualitative analysis was conducted by comparing the mass spectra of each individual analyte with those reported in the National Institute of Standards and Technology (NIST) database and by calculating linear retention indices for each analyte as well as by comparing them with the data reported in the literature [4]. Finally, when necessary, the phytochemical characterization was carried out by co-injection of reference standards. The semi-quantitative analysis was calculated as the percentage composition of each individual analyte of the total composition of the EO. Qualitative and semi-quantitative analyses allowed to obtain the characteristic profiles of the 4 EOs selected for this study. Subsequently, the antibacterial activity of the 4 EOs was determined against two *S. agalactiae* strains isolated from vaginal swabs. Initially, by agar diffusion method, sterile disks impregnated with the EO were placed on Tryptic Soy Agar previously seeded with the same bacterial strain. EOs bacterial growth inhibitory activity was highlighted by the absence of microorganism growth around the spot. Moreover, the Minimum Inhibitory Concentration (MIC) of the EOs individually and in association with Amoxicillin was tested against isolated bacterial strains. Amoxicillin was chosen since it is used to treat infections of *S. agalactiae* and for the MIC evaluation the agar dilution plating method was used. The last test performed was the microliter dilution plate to check the ability to reduce and/or inhibit the growth of *S.agalactiae* by individual EO and mixtures between two EOs. By using the agar diffusion method and considering the zone of inhibition, it was possible to verify the EOs inhibitory activity on the two strains of *S. agalactiae* examined. MIC results showed that the bacterial strains are sensitive to the antibiotic Amoxicillin, while the single EO and the association between them are not able to significantly decrease, at low concentrations, the growth of the two microorganisms. Excellent results were obtained in the case of association between Amoxicillin and EOs: such unions allowed to substantially decrease the MIC values with respect to the single antibiotic MIC value. Microdilution test results showed good synergistic activity between EOs, in particular lavandin and savory, savory and oregano, savory and thyme. The results show how the EOs studied have antibacterial activity against the two strains of *S. agalactiae* tested. In light of all the above and while expecting a clinical use of these EOs, an important data underlined by the phytochemical analysis is the phenolic monoterpenes high percentage, specifically in: savory, oregano, and thyme. This class of compounds is potentially toxic in high doses. Certainly, for a use at high dosages, according to gas chromatographic analysis, *Lavandula x intermedia* EO is the best choice since it presents no documented side effects. Another interesting outcome is that, despite the EOs low antibacterial activity at low concentrations obtained, EOs are able, in combination with the Amoxicillin, to generate a synergistic effect achieving significant reduction in the dose of the antibiotic and therefore limiting its potential toxic effects or side effects. The phytochemical analysis, in conjunction with the *in vitro* microbiological test, consented to obtain noteworthy EOs composition-antimicrobial activity correlations. All of these findings are definitely an advantage in the attempt to overcome the drug resistance emergency in a period like this, where even the most recent synthetic antimicrobials are not enough for multidrug-resistant bacteria. Conclusively, a valuable and helpful increased use of such natural extracts in the medical and pharmaceutical fields starts to occur, for example in gynaecology and obstetrics for the prevention and treatment of various symptoms and conditions; in clinical dermatology and surgery for the treatment of wounds and skin lesions, even in presence of microbial strains resistant to conventional drugs; finally, in the pharmacological and cosmetic industry for the production, also for preventive purposes, of detergents for personal hygiene, ointments, creams, ovules.

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ABSTRACTS DEGLI INTERVENTI IN ITALIANO

ATTIVITÀ BIOCIDA DEGLI OLI ESSENZIALI DI SPECIE DI MONARDA NEI CONFRONTI DI NEMATODI FITOPARASSITI

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I nematodi sono parassiti delle radici di un'ampia gamma di colture agrarie e a livello mondiale sono causa di circa il 14% delle perdite di produzione agricola, pari a circa 100 miliardi di dollari per anno.
I nematodi galligeni del genere *Meloidogyne* sono le specie più comuni e più distruttive, a causa dell'ampia gamma di piante ospiti (circa 2000 specie sia erbacee che arboree) e della loro presenza nelle aree temperate e calde di tutto il mondo.
Gli oli essenziali (OE) di molte specie di piante aromatiche e medicinali sono già stati segnalati per un'attività nematocida sui nematodi galligeni. La ridotta persistenza e la limitata tossicità rendono gli OE dei buoni candidati per la formulazione di nuovi nematocidi a basso o nulla impatto ambientale e quindi sostenibili per l'uomo e l'ambiente.
Sono state realizzate prove preliminari di laboratorio per valutare il potenziale nematocida di OE di *Monarda fistulosa* e *M. didyma* (al primo di anno di crescita in campi sperimentali allestiti ad Imola, sede distaccata dell'Università di Bologna) sul nematode galligeno *Meloidogyne incognita*. Le larve infettive di *M. incognita* sono state messe a contatto per 4, 8 o 24 ore con soluzioni acquose dei due oli a concentrazioni di 3.12, 6.25, 12.5, 25.0, 50 and 100 mL mL^{-1}. Già dopo sole 4 ore di esposizione alla concentrazione di 3.12 mL mL^{-1}, la mortalità larvale è risultata pari a quasi il 36% per l' OE di *M. fistulosa* e di circa il 29% per l'OE di *M. dydyma*. Alla concentrazione di 100 mL mL^{-1} di entrambi gli OE sono risultate morte circa il 78% delle larve. Una esposizione di 24 h alla concentrazione di 12.5 mL mL^{-1} di entrambi gli OE è stata sufficiente a determinare tassi di mortalità superiori all' 80%. In generale i tassi di mortalità sono risultati correlati maggiormente alle concentrazioni piuttosto che ai tempi di esposizione, senza mai mostrare differenze significative tra i due OE.
Questi risultati preliminari sembrano indicare che gli OE di *M. fistulosa* e *M. didyma*, entrambi caratterizzati da un'elevata concentrazione di composti fenolici, fra cui il timolo, sono in grado di svolgere una elevata attività nematocida esembrano dunque offrire buone possibilità d'impiego nella formulazione di nematocidi innovativi.

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EFFETTI SULLA CRESCITA E FORMAZIONE DI BIOFILM DI *STAPHYLOCOCCUS AUREUS* DOPO BREVE E PROLUNGATA ESPOSIZIONE AL CARVACROLO

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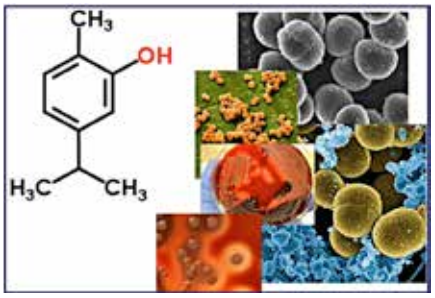
Introduzione

Il carvacrolo, componente importante di molti oli essenziali, tra i quali olio di origano e timo, possiede attività antimicrobica ad ampio spettro nei confronti sia di patogeni clinici che alimentari. Studi recenti documentano la sua efficacia nei confronti di ceppi di *Staphylococcus aureus* meticillino-resistenti, sia in fase planctonica (Nostro et al., 2004) che sessile (Nostro et al., 2009), anche dopo rilascio da sistemi "delivery" a base di etilen vinil acetato (Nostro et al., 2012). Nonostante i numerosi studi presenti in letteratura, poche evidenze sperimentali dimostrano l'impatto di esposizioni continue agli oli essenziali o loro componenti sulla sensibilità agli agenti antimicrobici e sulle caratteristiche di virulenza microbica (Hammer et al., 2012). Scopo di questo studio è quello di ampliare le conoscenze in questo campo e valutare gli effetti su *S. aureus* dopo breve e prolungata esposizione al carvacrolo.

Metodi

Gli esperimenti sono stati condotti su ceppi di *S. aureus* meticillino sensibili (*S. aureus* ATCC 6538) e meticillino-resistenti (*S. aureus* ATCC 43300 e *S. aureus* 815 isolato clinico). Colture overnight sono state esposte serialmente ogni 24 h a concentrazioni subinibenti di carvacrolo (0,5 x concentrazione minima inibitoria, MIC) per 3 (esposizione breve) e 10 (esposizione prolungata) giorni. Le colture adattate sono state successivamente sottoposte a passaggi seriali su terreno privo di carvacrolo. Sui campioni



di tutti gli stadi e sui controlli (batteri non adattati) è stata, quindi, determinata la sensibilità al carvacrolo, gentamicina, linezolid, mupirocina, norfloxacina e rifampicina secondo le linee guida CLSI, e sono stati valutati gli effetti sulla crescita planctonica e sulla formazione di biofilm mediante determinazioni spettrofotometriche. Sono stati, inoltre, eseguiti studi sulla morfologia delle colonie e sull'attività emolitica e coagulasica.

Risultati

I risultati di questo studio documentano che i ceppi di *S. aureus* meticillino-resistenti (ATCC 43300 e 815) sottoposti ad esposizioni brevi e prolungate al carvacrolo manifestano una sensibilità di poco inferiore al carvacrolo e una sensibilità invariata nei confronti degli antibiotici. Il ceppo di S. aureus meticillino sensibile (ATC6538), invece, dopo prolungata esposizione (10 giorni) al carvacrolo, ha manifestato una sensibilità nettamente ridotta alla gentamicina. Per quanto riguarda gli altri parametri, mentre non vi sono state notevoli differenze nella crescita planctonica tra i campioni adattati ed i campioni non adattati, sono state riscontrate diversità nella formazione di biofilm. In particolare, S. aureus ATC6538 ha mostrato un discreto aumento della produzione di biofilm, con conseguente minore sensibilità al carvacrolo e la presenza di "Small Variant (SCVs)" ovvero colonie con diametro < 1 mm, prive di pigmentazione, attività emolitica e coagulasica. Caratteristiche che, tuttavia, sono in parte riacquistate dopo i passaggi seriali in terreno privo di carvacrolo.

Conclusioni

I risultati di questo studio hanno mostrato che l'esposizione breve e prolungata di S. aureus a concentrazioni subinibenti di carvacrolo determina differenze in sensibilità e produzione di biofilm che sono ceppo-specifiche.

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COMPOSIZIONE, TRASFORMAZIONE E ATTIVITÀ DI OLIO ESSENZIALE DA *ACHILLEA MARITIMA* (L.) EHREND. & Y.P. GUO *SSP. MARITIMA* DI SARDEGNA

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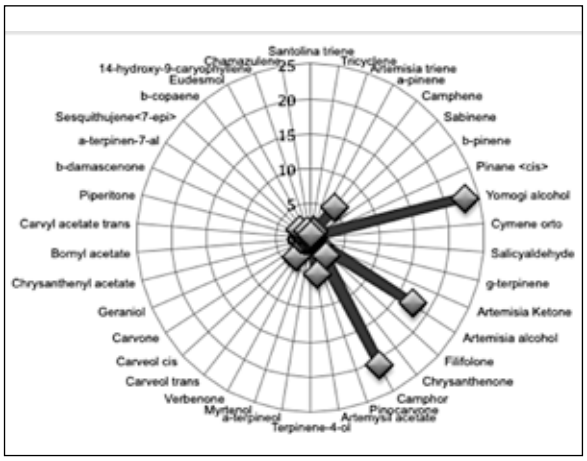
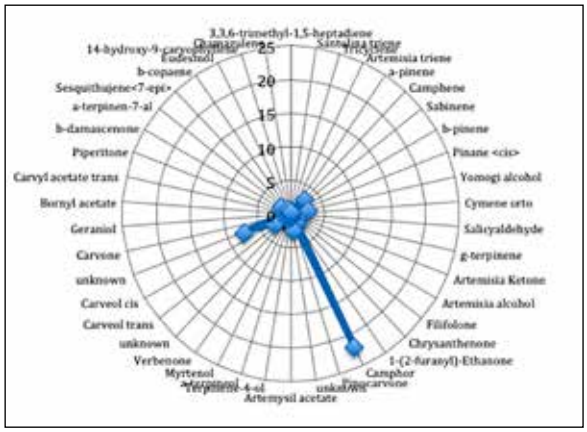
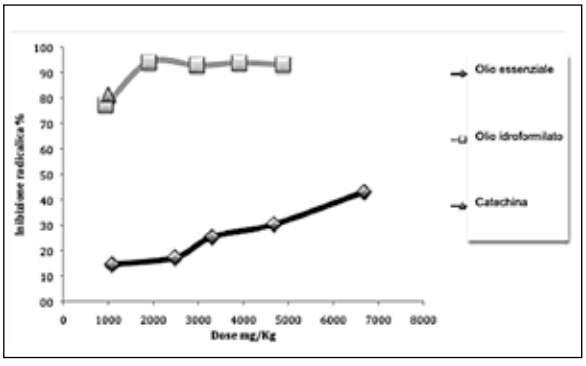
Il genere *Achillea*, proprio delle regioni temperate dell'emisfero boreale, appartiene alla famiglia delle *Asteraceae*. In Italia sono state individuate una ventina di specie spontanee distribuite su tutta la penisola, tra queste tipica del litorale sabbioso *Achillea maritima* (L.) Ehrend. & Y.P. Guo subsp. *maritima* pianta perenne, alta sino a 40 cm, aromatica, ricoperta da fitto tomento feltrato bianco, con vigorosi fusti ascendenti, leggermente lignificati, formanti un pulvino lasso; foglie sessili e semi-amplessicauli. Fiori con sole corolle tubulose di colore giallo, in capolini sferici con brattee involucrali rivestite di lanugine bianca, ricettacolo provvisto di squame, fiorisce da giugno ad agosto. Utilizzata sembra presentare una significativa attività biologica e farmacologica [1] Esistono pochi studi in letteratura sulla composizione chimica dell'olio essenziale di *A. maritima* o *Otanthus maritimus* ottenuto dalle parti aeree [2, 3] e non sono presenti studi sull'*A. maritima* vegetante in Sardegna, per questo motivo abbiamo raccolto del materiale vegetale spontaneo nella stazione di Badesi (Poltu biancu) dal quale è stato estratto l'olio essenziale in corrente di vapore. L'olio essenziale è stato caratterizzato. Sono stati identificati il 93% dei suoi costituenti. A eccezione della canfora, presente nella concentrazione del 20.8%, è evidente la presenza di costituenti monoterpenici irregolari quali: yomogi alcol (22.8%), artemisia alcol (17.4%) e artemisil acetato (5.4%).

Abbiamo dapprima misurato il potere antiossidante dell'olio e poi lo abbiamo sottoposto ad idroformilazione allo scopo di aumentare composti ossigenati con alto potere antiossidante. Le modificazioni chimiche prodotte dalla reazione d'idroformilazione hanno alterato le caratteristiche olfattive dell'olio, rendendolo più gradevole.

L'olio essenziale idroformilato è stato caratterizzato ed utilizzato per eseguire prove in vitro della capacità antiossidante. I principali prodotti che hanno subito l'idroformilazione sono stati: il canfene, lo yomogi alcol, l'artemisia alcol, l'artemisil acetato fornendo i rispettivi derivati composti carbonilici (E' stato modificato circa il 50% della composizione iniziale).







	Escherichia coli ATCC 8739	*Staphylococcus aureus* ATCC 6538	*Pseudomonas aeruginose* ATCC 9027	*Candida albicans* ATCC 10231
Othantus maritimus L. olio essenziale	> 2 mg/mL	2 mg/mL	> 4mg/mL	4 mg/mL (M.B.C. 4mg/mL)

L'incremento della capacità antiossidante, misurata *in vitro* (test DPPH) dell'olio essenziale idroformilato, è stato notevole arrivando ad attività superiori allo standard di riferimento come riportato nel grafico sottostante:

Inoltre è stato eseguito uno screening preliminare dell'attività antimicrobica utilizzando l'olio essenziale non modificato, i risultati sono stati non significativi sui ceppi testati, a eccezione di una modesta attività su *Staphylococcus aureus*. Nella tabella seguente sono riportate le MIC ottenute.

Da questo studio è risultato che l'olio essenziale di *A. maritima* ha una composizione particolare e una buona attività antiossidante, che viene fortemente incrementata se l'olio è sottoposto a trasformazione mediante idroformilazione. Dal punto di vista dell'attività antimicrobica i dati preliminari non hanno mostrato particolare efficacia sull'olio originale e dovrà essere verificata sugli oli modificati.

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CONTAMINAZIONE DI FRUTTI DI BOSCO DA PARTE DEL VIRUS EPATITE A: CARATTERIZZAZIONE DEL RISCHIO E VALUTAZIONE DELLE PROPRIETÀ ANTIVIRALI DEGLI OLI ESSENZIALI. RISULTATI PRELIMINARI

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Recenti evidenze epidemiologiche indicano che i virus enterici, in particolare il virus dell'epatite A (HAV) e norovirus (NoV), sono la principale causa di malattie di origine alimentare nei Paesi sviluppati (1, 5). Sebbene il consumo di molluschi bivalvi rimanga la principale causa di epidemie causate dai virus a trasmissione alimentare, i vegetali ed in particolare i frutti di bosco sono sempre più spesso riconosciuti come veicoli per questi patogeni. Negli ultimi anni le epidemie di HAV e norovirus legate al consumo di frutti di bosco congelati si susseguono sempre più frequentemente sia in Europa (200 casi in Finlandia, 11000 in Germania, 26 nel Nord Europa) (3), sia nel resto del mondo come USA, Canada e Hawaii (2). Anche in Italia il sistema di sorveglianza Seieva ha evidenziato un notevole incremento delle notifiche di epatite A (637 casi) nell'anno 2013 con una maggior parte dei casi riconducibili al consumo di frutti di bosco. Questi prodotti possono essere contaminati durante la coltivazione, prima del raccolto, oppure durante le varie fasi della lavorazione in seguito al contatto con lavoratori infetti, o con acque contaminate. I virus non si replicano nell'alimento ma hanno la capacità di resiste-

re per tempi lunghi tempi senza subire riduzione della carica infettiva (5) e dal momento che nella maggior parte dei casi la frutta e la verdura vengono mangiate crude, la contaminazione virale dei prodotti freschi rappresenta un importante rischio per la salute dei consumatori. I trattamenti comunemente utilizzati su questi prodotti (refrigerazione, congelamento, pH acidi, ecc.) risultano inefficaci nel rimuovere o inattivare completamente i virus. Il calore è la misura più efficace per la loro eliminazione, tuttavia i trattamenti termici come la cottura non sono sempre adatti alle caratteristiche commerciali del prodotto. Per questo motivo risulta interessante valutare trattamenti innovativi da applicare ai frutti di bosco senza alterarne le qualità organolettiche. L'applicazione degli oli essenziali nell'industria alimentare appare molto interessante, vista la loro attività antimicrobica e antivirale già nota in medicina umana; in letteratura negli ultimi anni sono comparsi lavori scientifici che testimoniano l'efficacia dei componenti chimici presenti negli oli essenziali su microrganismi patogeni (4). Le informazioni sulle contaminazioni virali ed i potenziali rischi per la salute risultano non bene documentate e fino ad oggi la mancanza di tecniche di rilevazione standardizzate ha reso difficile ottenere dati confrontabili. Il Ministero della Salute nel 2014 ha finanziato all'IZS Piemonte Liguria e Valle d'Aosta un progetto di ricerca che si prefigge di: monitorare la circolazione di HAV in frutti di bosco, valutare il rischio di contrarre un'infezione connesso al consumo di questo tipo di prodotto e valutare l'efficacia di trattamenti innovativi che non alterino le qualità organolettiche, come gli oli essenziali, per inattivare il virus dell'Epatite A.

Il progetto di ricerca è iniziato a dicembre 2014 e ad oggi sono stati analizzati 51 campioni di frutti di bosco surgelati, sia misti che in singola varietà, di provenienza internazionale. I campioni sono stati prelevati all'ingrosso presso ditte distributrici in Italia ed analizzati per la ricerca di HAV applicando la metodica di legge ISO/TS 15216-2.

Relativamente alla valutazione dell'attività antivirale degli oli essenziali sono stati inizialmente individuati gli oli essenziali da testare, in accordo alla loro composizione chimica e al fine di non interferire con le qualità organolettiche del prodotto del progetto di ricerca. Gli oli essenziali più adatti per questo scopo potrebbero essere quelli derivati da piante appartenenti al genere *Citrus* dal momento che la *Food and Drug Administation* li ha dichiarati GRAS (*Generally recognized as safe*) e che il loro profumo e sapore ben si adattano ai frutti di bosco e sono graditi al consumatore. Gli oli essenziali del genere *Citrus* contengono l'85-99% di componenti volatili e l'1-15% non volatili. I primi comprendono: monoterpeni (limonene), sesquiterpeni e idrocarboni, e i loro prodotti ossigenati che includono aldeidi (citrale), chetoni, acidi, alcoli (linalolo) ed esteri. In accordo a questi requisiti sono stati identificati: limone, arancio e pompelmo che verranno diluiti in idoneo vettore. Ad oggi sono stati effettuati test di tossicità per diverse miscele di vettore, utile per solubilizzare gli oli essenziali, su colture cellulari di linea Frp-3 sensibili all'infezione da HAV.

Sono state testate due serie di miscele da 8, ottenute con Tween 80 o DMSO in aggiunta a etanolo ed olio di arachide. A tutte le miscele è stato aggiunta una soluzione antimicotica/antimicrobica più due soluzioni di bianco (Tab.1). Otto miscele, identificate come gruppo A, sono state messe a contatto con il tappeto cellulare per 1h e quindi allontanate; il tappeto cellulare è stato lavato 3 volte con PBS e si è proceduto poi con le normali tecniche colturali con il 3% di siero e osservate per 4 giorni. La linea cellulare è stata osservata al microscopio a 1h, 5h, 12h, 3gg, 4gg. Otto miscele, identificate come gruppo B, sono state messe a contatto con il tappeto cellulare per 1h e successivamente è stato aggiunto il terreno di coltura con il 3% di siero e si è proceduto poi con le normali tecniche colturali con il 3% di siero. La linea cellulare è stata osservata al microscopio a 1h, 5h, 12h, 3gg, 4gg.

Miscele	Composizione percentuale
1	65% soluzione fisiologica; 33% olio di arachide; 1% DMSO; 1% antibiotico/antimicotico
2	65% soluzione fisiologica; 33% olio di arachide; 1% TWEEN 80; 1% antibiotico/antimicotico
3	64,9% soluzione fisiologica; 33% olio di arachide; 1% DMSO; 1% antibiotico/antimicotico; 0,1%ETOH
4	64,9% soluzione fisiologica; 33% olio di arachide; 1% TWEEN 80; 1% antibiotico/antimicotico; 0,1%ETOH
5	65,9% soluzione fisiologica; 33% olio di arachide; 0,1% DMSO; 1% antibiotico/antimicotico
6	65,9% soluzione fisiologica; 33% olio di arachide; 0,1% TWEEN 80; 1% antibiotico/antimicotico
7	90% soluzione fisiologica; 10% antibiotico/antimicotico
8	90% soluzione fisiologica; 10% antibiotico/antimicotico

Tab. 1 Miscele vettore testate su colture cellulari linea Frp-3

Tutti i campioni di frutti di bosco, di provenienza internazionale, sono risultati negativi per la ricerca del virus dell'Epatite A. Dai test di tossicità per le miscele vettore è emerso che il gruppo A generalmente ha mostrato meno sofferenza cellulare, sebbene al quarto giorno di trattamento le cellule abbiano cominciato a mostrare indici di sofferenza dovuti probabilmente alla confluenza e non al trattamento con la miscela. Il gruppo B invece ha mostrato generalmente più sofferenza; in particolare nei tappeti cellulari trattati con la miscela 2 e 4 si denotano molti detriti cellulari e contorno cellulare sempre più necrotizzato. Al quarto giorno di trattamento però nessun tappeto cellulare ha mostrato mortalità. Questi dati sono da considerarsi preliminari e solo al termine dei tre anni, previsti per lo svolgimento del progetto di ricerca, si potranno avere maggiori informazioni relativamente alla caratterizzazione del rischio e per le proprietà antivirali degli oli essenziali selezionati.

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CARATTERIZZAZIONE DI OLIO ESSENZIALE DI THYMUS VULGARIS L. DA DIVERSE ACCESSIONI PROVENIENTI DA COLONIE SPONTANEE IN VALLE D'AOSTA

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Nell'ambito della valorizzazione delle risorse del territorio, in Valle d'Aosta in questi anni sta aumentando l'interesse per la coltivazione di specie spontanee autoctone, in particolare delle piante aromatiche e officinali [1]. La caratteristica conformazione orografica ed il clima favorevole della regione fanno sì che essa rappresenti il limite settentrionale dell'area di diffusione spontanea del *Thymus vulgaris* L., che risulta una specie interessante per le zone marginali [2]. Questa pianta è nota fin dall'antichità per le sue molteplici proprietà biologiche e farmacologiche e, da un'indagine di mercato condotta in precedenza dal nostro Istituto, è risultata una specie estremamente interessante per le sue potenzialità commerciali [3].
Il seguente lavoro è finalizzato alla determinazione della composizione chimica dell'olio essenziale di *T. vulgaris* L., proveniente da popolazioni valdostane, allo scopo di confermare la sola presenza del chemiotipo a timolo [2]. Tutto questo ha l'obiettivo di valorizzare il patrimonio genetico locale, salvaguardando la biodiversità del territorio regionale, e di incrementare l'interesse di coltivatori e consumatori verso una specie, tradizionalmente conosciuta, dalle molteplici potenzialità non ancora pienamente espresse.
Nel 2011 sono state individuate 6 popolazioni di questa specie, tutte situate sul versante solatìo della vallata principale ma ad altitudine differente. I semi raccolti in estate sono stati seminati in alveoli durante l'autunno. Dopo l'inverno in serra, nella primavera 2012 è avvenuto il trapianto in pieno campo delle 6 accessioni e della varietà commerciale "Varico 2", a chemiotipo timolo, scelta come confronto. Il campo sperimentale è stato organizzato in modo da avere 5 ripetizioni per ciascun campione. Negli anni 2013 e 2014 le sommità fiorite sono state raccolte alla piena fioritura ed essiccate in un essiccatoio tradizionale. L'olio essenziale, ottenuto per distillazione in corrente di vapore dell'essiccato, è stato posto in contenitori di vetro scuro e conservato in frigorifero fino ad analisi. La sua caratterizzazione, sia qualitativa che quantitativa, è stata eseguita in triplo con le tecniche GC-MS e GC-FID utilizzando in entrambi i casi la stessa colonna e le stesse condizioni cromatografiche.
In tutti gli oli essenziali analizzati sono stati identificati 22 composti chimici che rappresentano il 96% circa dell'intera frazione volatile e, fra essi, i fenoli sono risultati i componenti più abbondanti. Il timolo è stato rilevato in percentuale media superiore al 35%, considerando tutti i campioni analizzati nei due anni. Si sono osservate delle differenze fra le accessioni e solo due di queste hanno mostrato una percentuale in timolo media, su due anni, del 49%, uguale a quella riscontrata nel campione commerciale. In questi due campioni sono state rilevate anche delle alte concentrazioni di precursori biosintetici del timolo, in percentuali che vanno dal 14% al 22% per il *p*-cimene e dal 12% al 17% per il γ-terpinene.
In base ai risultati riportati possiamo confermare che *T. vulgaris* L. che vive nei siti individuati sul territorio valdostano è a chemiotipo timolo. Vista la sua composizione quali-quantitativa, si conferma il notevole interesse farmaceutico della specie e si auspica, in un futuro, di selezionare le due varietà locali che si sono dimostrate più ricche in questo principio attivo e di avviarne la coltivazione. Tale prospettiva andrebbe incontro alle richieste provenienti dai settori commerciali valdostani fra cui quello erboristico-farmaceutico e quello cosmetico [3].

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COMPOSIZIONE CHIMICA E ATTIVITÀ BIOLOGICA DI OLI ESSENZIALI DA *HELICHRYSUM ITALICUM* SSP *MICROPHYLLUM* (WILLD.) NYMAN DEL NORD SARDEGNA

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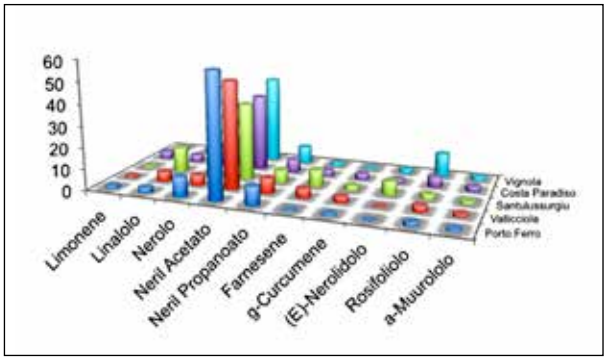
Il genere *Helichrysum* (*Compositae*) comprende 16 specie ed è rappresentato da erbe o arbusti nani, spesso lanate o tomentose. Il nome Helichrysum proviene da due parole greche Elios e crusòs che significa sole d'oro. I capolini sono di piccola o di media grandezza con fiori gialli tutti tubulosi (1). Diverse specie sono ampiamente rappresentate nella flora mediterranea. I primi dati relativi agli oli di Helichrysum sono stati pubblicati da Gildemeister (2); la specie maggiormente diffusa in Sardegna allo stato spontaneo è *Helichrysum italicum* (Roth) G. Don ssp. *microphyllum* (Willd.) Nyman.
Helichrysum italicum è noto per avere alcune proprietà interessanti, infatti suoi estratti sono: anti-infiammatori, antiallergici, anti-eritema e fotoprotettivi. Inoltre può essere utilizzato per le sue proprietà balsamiche, bechiche, è ampiamente usato nel preparato cosmetico come dopo-sole, è utile inoltre a uso esterno per la psoriasi (3). Per tutti questi motivi abbiamo deciso di

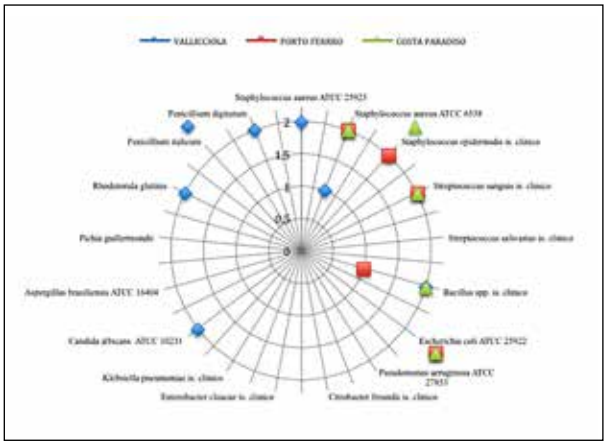
studiare la variazione tra i principali costituenti dell'olio essenziale di *H. italicum* ssp. *microphyllum* vegetante spontaneo nel nord Sardegna. L'obiettivo era quello di monitorare l'eventuali variazioni di composizione chimica degli oli essenziali e di valutarne l'attività antimicrobica. Nella tabella sottostante sono riportate la localizzazione, le caratteristiche pedologiche e la resa in olio essenziale dei campioni analizzati.
Tra i 46 costituenti individuati nell'olio essenziale solo 10 sono

Stazione	Localizzazione ed altitudine	Caratteristiche pedologiche	Resa %
Porto Ferro	Sardegna Nord-occidentale, stazione localizzata in prossimità del litorale, altezza slm 20m	Terreno evoluto su sabbie eoliche pH neutro e sub-alcalino, sabbioso	0.18
Vignola	Costa settentrionale stazione localizzata su un terreno collinare con esposizione S.-S.E. 120 m slm	Terreno evoluto su rocce intrusive, graniti e granodioriti, pH sub-acido, franco sabbioso, buona permeabilità.	0.18
Costa Paradiso	Costa settentrionale, stazione localizzata in prossimità del litorale, 80 m slm.	Terreno evoluto su rocce intrusive, graniti e granodioriti, pH sub-acido, franco sabbioso, buona permeabilità.	0.20
Santulussurgiu	Sardegna nord-orientale, ampia stazione che si sviluppa ai piedi del Limbara,	Terreno evoluto su rocce effusive basiche, basalti, pH neutro. Franco-argilloso.	0.18
Valliciola	Sardegna nord-orientale, stazione localizzata sulla sommità del Limbara, 800 m slm.	Terreno derivante dalla disgregazione di rocce granitiche, pH acido sub-acido.	0.20



presenti in alta quantità ed alcuni sono peculiari delle singole popolazioni. Tutti gli oli essenziali sono caratterizzati da un alto contenuto di neril acetato che varia tra il 58.2% ed il 35.9%. L'elicriso che cresce a Porto Ferro è quello più ricco di questo componente, mentre la percentuale più bassa la ritroviamo nei campioni di Santulussurgiu e Costa Paradiso (35.9%; 36.5%).
Il nerolo raggiunge una concentrazione dell'10.9% nell'elicriso proveniente da Costa Paradiso e una percentuale tra il 9-10% nei campioni di Santulussurgiu, Porto Ferro e Vignola.
Il più basso contenuto in nerolo lo abbiamo riscontrato negli oli essenziali dell'elicriso di Vallicciola.
Per quello che riguarda la concentrazione del neril propionato vediamo la concentrazione maggiore (8.8%) nei campioni raccolti a Porto Ferro, mentre la sua concentrazione minore (5.9%) è presente in quelli di Santulussurgiu. Il linalolo mostra una estrema variabilità andando da un massimo dell'11.6% (Santulussurgiu) ad un minimo del 2.3% (Porto Ferro).

Gli oli essenziali derivanti da Porto Ferro, Vallicciola e Costa Paradiso sono stati utilizzati per uno screening antimicrobico; poiché questa attività può provenire da composti monoterpenici ossigenati quali neril acetato, acetato di geranile, geraniolo, e nerolo (4) sono stati selezionati i due oli a maggior concentrazione di neril acetato e neril proprionato e quello a minor concentrazione di questi due costituenti.
Le minime concentrazioni inibenti (MIC) sono state determinate mediante tecniche standard di diluizione in agar (Mueller Hinton Agar per i batteri, Sabouraud Dextrose Agar per i miceti).
Tra i vari microrganismi abbiamo ritenuto interessante utilizzare anche *Pichia guillermondii*, un lievito presente sulla cute umana e su molti alimenti, che può provocare infezioni cutanee in pazienti immunocompromessi e *Rhodotorula glutinis* lievito ubiquitario nell'ambiente che può provocare infezioni in pazienti immunodepressi.



Come riportato nel grafico, l'attività dimostrata da questi oli essenziali non è risultata molto efficace, si nota però una discreta attività contro *Staphylococcus aureus* ATCC 6538 da parte dell'olio essenziale di Vallicciola e contro il *Bacillus* spp. is. clinico da parte dell'olio essenziale di Porto Ferro. Inoltre si è verificata una blanda attività su altri ceppi come riportato nel grafico. I test confermano che l'attività è derivante fondamentalmente dai monoterpeni ossigenati, infatti l'olio essenziale di Costa Paradiso, a minor contenuto di questi costituenti, non ha mostrato attività significativa sui ceppi utilizzati.

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