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1968-2008: 40 Years of Franco F. Vincieri's Natural Products Research

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This paper presents an overview of Prof. Vincieri's accomplishments in his career as a researcher in the field of pharmacognosy (pharmaceutical biology), analytical phytochemistry and pharmaceutical technology applied to herbal drug preparations at the Department of Pharmaceutical Sciences of the University of Florence. This article is a recognition of his valuable contributions to these research fields, especially for his outstanding and innovative interdisciplinary studies on the quality control of herbal drugs, herbal drug preparations, herbal medicinal products, botanical food supplements, and some "special foods" such as grapes, wines, olives and olive oil.

Keywords: Franco Francesco Vincieri, Department of Pharmaceutical Sciences, University of Florence, pharmacognosy (pharmaceutical biology), analytical phytochemistry and pharmaceutical technology, herbal drug preparations.

This issue of Natural Product Communications is dedicated to the 70th birthday of Franco Francesco Vincieri, Full Professor at the University of Florence, Department of Pharmaceutical Sciences. It has been my special honor to prepare this paper concerning some of his outstanding achievements in the field of pharmacognosy (pharmaceutical biology), analytical phytochemistry and pharmaceutical technology applied to herbal drug preparations, especially for his important and innovative interdisciplinary studies on the quality control of herbal drugs, herbal drug preparations, herbal medicinal products, botanical food supplements and some "special foods" such as grapes, wines, olives and olive oil. I am grateful for the comments and contributions of all colleagues, former and present students, and friends who participated in this special issue for their comments and contributions, and in particular for the construction and revision of this article. My special thanks to Anastasia Karioti for her patient collaboration in reading and commenting on some parts of the paper.

Scientifically Prof. Vincieri is a very polyhedral and curious scientist, "a sort of active vulcan", ready to start a new experience, even if it is an expedition to the African desert (Figure 1). Most of his success has come from his own intense efforts and his extreme versatility and passion for research, which led to his many accomplishments, awards and memberships which highlight his extraordinary abilities, not only as a scientist, but also as a person.

Figure 1: Franco duing an expedition through Sahara from Algeria to Giordania. The Sheik, Jeleil al-Deisah, with one of his wives, Hadra, and his childrem is recognising some herbal drugs collected by Franco (in the middle) and his assistent Giacalone (on the right).

For these reasons, in 1997, when as a post-doc I moved from the University of Pisa for a permanent position as Researcher at the Department of Pharmaceutical Sciences in Florence, he has had a deep personal and professional impact on my life, a sentiment that is shared by all his former and present students, some of whom are now either post-docs or colleagues in this Department.

Figure 2: ESCOP meeting at Hailsham (UK) in June 1995. Franco talking to the late Prof. Hein Zeylstra (on the left of the picture) and Prof. Finn Sandberg.

Among his publications, including several chapters and books (most of them used as text books for undergraduate, postgraduate, master's and PhD students), I have selected papers concerning several projects which represent the scope and breadth of his work. In addition, these publications are considered milestones for the development of many scientific activities, not only of his research group but also of the Department of Pharmaceutical Sciences, which attracted many young researchers from all parts of the world, and led to finance for his work from private and public pharmaceutical companies. His firm belief that both basic and applied research are equally important is reflected in his efforts in 1981 when he was instrumental in organizing the Italian Society of Phytochemistry (Società Italiana di Fitochimica, SIF), an association of both university and company researchers. He was for many years the Italian delegate of the EMEA (the European Medicines Agency) and ESCOP (the European Scientific Cooperative on Phytotherapy) Scientific Committee working to assess criteria for efficacy and safety of herbal medicinal products in Europe.

At the beginning of his academic career (in the late Sixties-early Seventies) he worked with his colleague and friend, Prof. Massimo Bambagiotti Alberti (the current Director of the Department), on the analysis of terpenoids and related volatile compounds which marked the starting point for their ever-increasing interest in separation science. After a few years, Prof. Silvia Coran joined them, and later on Prof. Gloriano Moneti and Prof. Valerio Giannellini. In a short timethe group became well known at the University of Florence for their modern analytical chemistry, thanks to the introduction of the first GC- MS at the University of Florence. There are several papers [1-7] related to volatiles from *Pinus* species using diverse spectroscopic approaches for the structural elucidation of the components In the same period his friendship and collaboration [8,9] with Prof. Sergio Pinzauti, today the Dean of the Faculty of Pharmacy, also began.

Figure 3: *Sedum telephium* L. ssp. *maximum* Schinz & Thell.

Prof. Vincieri's instrumental ability attracted research groups of the University of Florence interested in diverse disciplines, and in particular Prof. Maria Teresa Vincenzini, one of the most outstanding scientists in Florence, interested in the effects of some natural constituents on the biochemistry of enzymatic systems (dehydrogenases, lyases), especially on germination of several medicinal plants. Their collaboration was confirmed by many papers published during the Sevetnties [10-16].

The studies of *Sedum telephium* L. ssp. *maximum* Schinz & Thell. and *Oenanthe aquatica* (L.) Poiret marked the beginning of analysis of natural constituents from the plant kingdom for the Department, in addition to the analysis of pharmaceutical products.

S. telephium (Crassulaceae) (Figure 3) is largely diffused in traditional medicine, especially in Tuscany, as a remedy for the local treatment of wounds and inflammatory diseases of the skin [17]. The term "telephium" is probably related to its vulnerary properties, introduced by Plinius, who first reported this plant as the herbal drug used to cure the leg wounds of King Telephium. The leaves (without the external cuticle, Figure 4) are reported in "The medicatis herbarum facultatibus" by Fulgenzo Vitman (1770), a monk of Vallombrosa (Florence), with this description: ***"Ulcera detergit***...(clean ulcers)***...et ad cicatricem perducit*** ... (and support

Figure 4: Preparation of *Sedum* leave before its application.

cicatrization) ***..tumorum suppurationem promovet ...*** (favour the suppurative phlogistic process) ***.. et dolores mitigat*** (and sooth pain)".

At the beginning of the Eighties, the properties of either the fresh or deep-frozen leaves, with the peel of the inferior part removed, were rediscovered and confirmed by a clinician, Dr Sergio Balatri, first at the Emergency Unit of the Torte Galli Hospital and later at San Giovanni di Dio Hospital, Florence in the treatment of various local inflammatory conditions, including whitlow, abscesses, complicated wounds, burns, poor cicatrisation, cysts, ulcerous phlebitis, and horniness (Figure 5) [18].

Figure 5: Application of the fresh peeled leaves of *Sedum telephium* to a withlow resulting in rapid healing, shown in "before" and "after" pictures.

Dr Balatri put forward to Prof. Vincieri the need for chemical analysis of the plant, the testing of its extracts to recognise the compounds responsible for the activity, and the preparation of modern formulations for use in the clinic. Studies started with the degree thesis investigations of Nadia Mulinacci [19], currently associate professor in the Vincieri's team, and collaboration with Prof. Hildeberg Wagner (University of Munich, Germany). This led to the isolation and identification of polysaccharides from the leaf tissue which had anti-inflammatory potential, including an anticomplementary effect *in vitro*, induction of TNF-alpha-production, increasing phagocytosis *in vitro* and *in vivo* [20]. Two years later the flavonoids were also identified, including two new ones, kaempferol-3-*O*-β-neohesperidoside-7-*O*-α-rhamnoside and quercetin-3-*O*-β-neohesperidoside-7-*O*-α-rhamnoside, which could contribute to the anti-inflammatory activity of *Sedum* [21].

Negative-ion fast-atom bombardment mass spectrometry was employed in the identification of flavonol glycosides directly in the juice [22]. Other methodologies were proposed for the qualitative and quantitative determination of flavonol glycosides, in particular a study in collaboration with Prof. Hermann Stuppner and coworkers (University of Innsbruck, Austria), which used MEKC analysis which was compared with HPLC-MS using electrospray ionization (ESI) interface [23]. In another collaborative study with Prof. Francesco Bonina (University of Catania, Italy), *in-vitro* and *in-vivo* studies suggested that, both the total lyophilized juice and, in particular, the lyophilized flavonoidic fraction, but not the lyophilized polysaccharidic fraction of the leaves, have photoprotective effects against UVB-induced skin damage [24]. Further studies with Prof. Renato Pirisino, Prof. Laura Raimondi and Prof. Maria Grazia Banchelli of the Department of Preclinical and Clinical Pharmacology of the University of Florence revealed that total *Sedum* juice strongly inhibited cell adhesion to laminin and fibronectin (EC50 1.03±0.12 mg mL^{-1}). This anti-adhesive feature was concentrated mainly in the two polysaccharide fractions (EC_{50} values between 0.09 and 0.44 mg mL^{-1}). The flavonol fractions did not seem to contribute to this effect [25].

According to the phytochemical, biological and pharmacological findings, some simple preparations were developed using either the fresh or lyophilized juice, and fractions. However, in contrast to the fact that extracts can represent the best way to select constituents of plants to be used for medicinal purposes, in the case of *Sedum* it was not possible to obtain the active fraction or constituents and prepare a valid formulation from them. The leaves still represent the best form for application, as a natural plaster. All efforts to update this simple formulation have been fruitless.

Another interesting medicinal plant investigated by Prof. Vincieri is *O. aquatica* (Apiaceae, Figure 6). Fruits, their alcoholic extract and essential oil are widely reported as a valuable remedy for dyspepsia, for the treatment of chronic pectoral affections as an

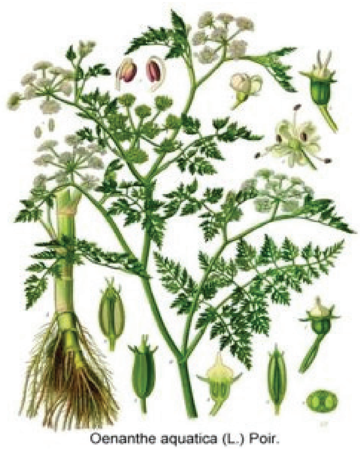

Figure 6: *Oenanthe aquatica* (L.) Poiret.

expectorant, for intermittent fevers, as a diuretic and for obstinate ulcers [26]. The infusion of the fruits was also reported by the "Farmacopea italiana del Regno" until the 4th Edition (1920). However, it has been known for a long time that the fruits can cause vertigo, dizziness, inebriation, dull pains in the head and other narcotic effects, as reported in "A Further Account of the Poisonous Effects of *Oenanthe aquatica* Succo Viroso Crocante of Lobel, or Hemlock Dropwort" [27].

Problems related to this herbal drug and preparations were diverse, all related to its safe use. Initial studies by Prof. Vincieri provided definitive information on the composition of the essential oil and the light petroleum (40-60°) extract of the fruit by a combination of different techniques: GLC, UV and IR spectroscopy and MS [28]. A group of C_{15} hydrocarbon and oxygenated polyacetylenes, including three new compounds, were also isolated and structurally identified on the basis of spectroscopic evidence. Due to their extreme sensitivity to air, heat and light, removal of the solvents and spectroscopic manipulation of the sample was performed by a home-made device to directly measure NMR spectra, similar to the modern concept of the hyphenated systems. IR spectra were performed in the crystalline state at liquid nitrogen temperature to observe the out-of-plane vibration band of the *cis*-double bond which, in the liquid phase, forms one broad band with the -CH_2-rocking vibration [29,30]. With the aim of finding a rapid method of characterization of polyacetylenes, second-derivative UV spectra of polyacetylenes were studied with Prof. Mario Pio Marzocchi and Prof. Giulietta Smulevich, colleagues in the Department of Chemistry of the University of Florence. This method was useful for the complete structural identification of their chromophores, including the stereoisomerism of the double bonds [31]. The second-derivative technique was also applied to a series of related naturally-occurring polyenynes, whose chromophoric fragments ranged from three to six conjugated groups. These results account for the structural characteristics, including steroisomerism, of a given sequence of triple and double bonds providing a complete fingerprint of the polyenyne chromophore [32]. In another paper, the application of the MS-MS technique for the rapid monitoring of some γ-butyrolactone ring and related lignans of *O. aquatica* fruits infusion was reported [33]. Finally, the GC-MS technique led to the identification of the constituents of the fruit tincture.Ten polyacetylenes, three lignans (derivatives of matairesinol), numerous monoterpenes, including phellandrene and cryptone, dillapiole and small amounts of sterols were identified [34]. Due to the low stability of the constituents of the tincture, especially polyacetylenes, a novel system of stabilization of the active principles of tinctures by means of cyclodextrins (α, β, γ-CyD) was also proposed. Microinclusion of the components was found to be incomplete with all three cyclodextrins, however, β-CyD was the most efficient and the stabilization of the most unstable microincluded active principles was verified by means of artificial ageing studies. These studies were possible thanks to an expert pharmaceutical technologist, Prof. Giovanni Mazzi, who joined the Vincieri group during that period. Further studies through artificial membranes also provided evidence for an increase in the permeability of the constituents. These studies on *O. aquatica* represent a good example of an interdisciplinary approach, including the enhanced biopharmaceutical properties of the formulated phytocomplex, representing a true milestone in this field of research [35,36].

During the 1980s-1990s, Vincieri expanded his chemistry program to cover many other herbal products and in particular vegetal matrices of interest in the biological and/or alimentary fields aiming at the development of specific methods of extraction, fractionation, isolation and characterization of potentially interesting secondary metabolites for the pharmaceutical, alimentary and cosmetic fields. Two typical Tuscan species with agro-alimentary interest, *Vitis vinifera* L. (leaves, fruits, wines) and *Olea europaea* L. (leaves, olives, oil, olive residues and waste waters), have been included in his investigations. Other plants belonging to the Mediterranean maquis, such as *Myrtus communis* L.,

Figure 7: Sangiovese, a typical grape variety of Chanti DOCG wine.

Pistacia lentiscus L., *Phillyrea latifolia* L., *Ligustrum vulgare* L., *Ligustrum sinensis* L., *Fraxinum ornus* L., *Arbutus unedo* L. were also studied [37-39].

Studies of polyphenols in wines and grapes were developed by Dr Alessandro Baldi and Prof. Annalisa Romani, former PhD students, and Romani later as a post-doc, researcher and associate professor. The key studies are represented by the development of an analytical method for anthocyanins of *Vitis vinifera* L. (Vitaceae) [40,41].The pool of anthocyanins contained in the berry skins of different cultivars of *V. vinifera* was taken as a research model to investigate the possible application of HPLC/MS to anthocyanins. The interface chosen was the API (atmospheric pressure ionization) ion spray interface coupled with a quadrupole mass spectrometer, which allows ambient pressure ionization and the use of any aqueous eluent.The use of this technique made it possible to obtain the mass spectra of all the anthocyanin compounds present in the extracts under investigation, even those occurring in traces or some coeluted ones. Studies were first carried out on certificated clones belonged to two varieties commonly used for the production of the Chianti DOCG red wine: Sangiovese (clone SS-F9-A5-48, [40] and Colorino (clone Nipozzano 6) [41].

The developed HPLC method led to the identification of the 3-glucosides, the 3-acetylglucosides, and the 3-*p*-coumaroylglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, already known in the literature. Two 3-caffeoylglucoside derivatives were identified too, and for the first time, some 3,5-diglucosides. The investigated cultivars showed the same anthocyanin profile, but dramatic quantitative differences, i.e. the cultivar Sangiovese showed a lower amount of the acylated compounds. This analytical application was, therefore, useful as a supporting technique for the structural investigation

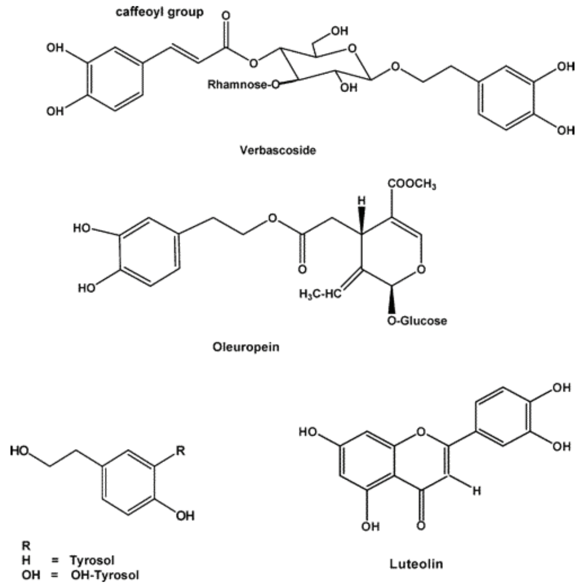

Figure 8: Structure of the characteristic phenols of *O. europea.*

of the polyphenolic compounds of different cultivars used in the production of red wines [40,41].

Numerous studies have been published on the analysis of olives, olive oil, waste waters (OMWW) and solid olive residue (SOR), experimental or commercial ones, from cultivars of different origins, stoned or whole fruits, and overall, there are more than 50 publications by the group up to now. However, in this case, I have selected some key studies carried out mainly by Prof. Nadia Mulinacci and Annalisa Romani, and more recently, by Prof. Patrizia Pinelli, as a graduate then PhD student, and as a postdoc. Olive oil is obtained from the olive (*Olea europaea* L., Oleaceae), a traditional tree crop of the Mediterranean Basin. On a European scale, 3 million tons of olives are processed for olive oil per year (with an oil yield of about 60,000 tons) [42]. After the epidemiological evidence of a lower incidence of CHD in the Mediterranean area [43] and certain types of cancers [44], there was an increasing popularity of the Mediterranean diet, in which olive oil is the major oil component, and its consumption is expanding to non producer countries such as the United States, Canada, and Japan. Olives and olive oil contain phenolic compounds, which not only influence the sensory properties, but are also important markers for type, biodiversity and quality determination of this product. These polyphenols have been shown to exert potent biological activities, including principally, but not limited to, antioxidant and free radical scavenging actions [42,45]. Some of the most representative phenolic compounds are hydroxytyrosol (3,4-dihydroxyphenylethanol),

Figure 9: Frantoio, a typical variety of Tuscan olive oil

tyrosol, oleuropein, verbascoside and luteolin and its derivatives (Figure 8). Studies were first focused on the extraction of the minor polar compounds from olive fruit [46] and from extra virgin olive oils [47,48]. A solid-liquid extraction (LSE) procedure (Extrelut cartridge, diatomaceous earth), followed by HPLC-DAD-MS analysis led to the characterization of the polyphenolic content of different Tuscan olive cultivars (Frantoio, reported in Figure 9, Rossellino, Ciliegino, Cuoricino, and Grossolana), including phenolic acids, verbascoside, oleuropein derivatives, flavons and flavonol glycosides [46].

Verbascoside was proposed as a chemotaxonomic marker of different cultivars. Numerous comparative studies [47,48] were also carried out on extra-virgin oils from different parts of Italy and obtained from several harvest years (1999-2002) from both stoned and whole fruits. A higher antioxidant capacity of the oils from stoned olives was found. At the end of the '90s, a project supported by the European Community entitled "Natural antioxidants from olive oil processing waste waters" (FAIR PL 973039) was commenced with the aim of evaluating the polyphenolic contents in different samples of olive mill waste waters (OMWWs), and the possibility of recovering them. It is well-known that OMWWs contain powerful pollutants [49] which are acidic (pH 5-5.5) and malodorous, containing potassium and phosphorus salts and organic substances, such as fats, proteins, sugars, organic acids, but also polyphenols. For this purpose a preliminary qualitative screening of the polyphenols was performed working on waste waters obtained from an experimental mill in Tuscany [50], noting antioxidant and other biological activities of extracts obtained from this matrix [51].

Extracts presented both phenolic polymers and low and medium molecular weight phenols such as elenolic acid, hydroxytyrosol, and tyrosol. Antioxidant and anti-inflammatory properties were proven. A study on experimental and commercial OMWW from four Mediterranean countries (Italy, Spain, France, and Portugal) [52] was also carried out. The results demonstrated that Italian commercial OMWWs were the richest in total polyphenolic compounds with amounts between 150 and 400 mg/100 mL of waste waters. These raw, as yet unused, matrices were found to be an interesting and alternative source of biologically active polyphenols.

A further work was aimed at investigating the phenolic content of another by-product, the solid olive residue (SOR). The aim of this investigation was the selection of the best extraction procedure to increase the yields of phenylpropanoidic derivatives in the obtained extracts [53]. Soxhlet extraction with ethanol was identified as the first step of purification and was followed by either a liquid/liquid extraction with ethyl acetate or fractionation using an ion-exchange resin. The total concentration of the phenolic compounds ranged between 1.1 and 6.23 mg/g of fresh SOR. Phenolic distribution among the different chemical classes was due to several factors: type of cultivar, degree of ripening, different milling processes, and pedoclimatic factors. The greatest differences among the samples were observed for verbascoside, which ranged between 0.15 and 4.15 mg g^{-1}, and for its less abundant analogues [53].

When in 1997 I joined the group of Prof. Vincieri, my research studies were mostly directed toward pharmaceutical and technological aspects of herbal drugs, herbal drug preparations and herbal medicinal products. The first person I met in the Department was Dr. Sandra Gallori, an expert technician with a deep artistic sense. She was able to transform a simple poster into a work of art and the Vincieri group has been known for a long time in the scientific community also for these artistic posters, obtaining several awards. In many occasions she has prepared leaflets for congresses. An example of her work is a special picture representing the Vincieri group as an anthill (Figure 10).

In 1998 Prof. Maria Camilla Bergonzi joined the group in order to start her PhD and during the following years, first as a post-doc and since 2005 as an aggregate professor, she has strongly supported my research. Authentication, quality control and stability testing have largely been performed using not only conventional [54-72] but also non conventional methods such as 1D- and 2D-NMR, NIR, and biosensors [73-77].

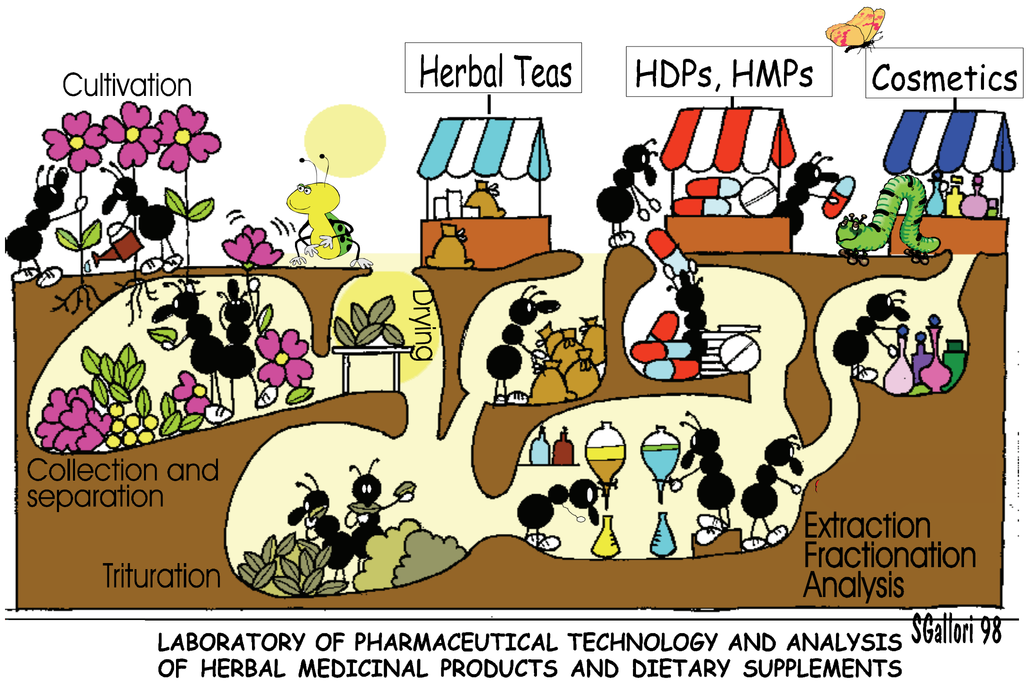

Figure 10: A portrait of Vincieri research team.

At the same time my scientific interest was driven by the improvement of bioavailability and technological features of extracts and HDPs, HMPs by formulation with liposomes, micelles, supramolecular complexes with cyclodextrins and their characterization by conventional methods (HPLC, DSC, UV, light scattering, dissolution tests) and innovative ones, such as NMR (ROESY and DOSY) [78-84].

Our publications represented the first studies on the stability and compatibility with excipients of important dried commercial extracts, such as St. John's wort (SJW), and results were dramatic when the studies were carried out using ICH guidelines [56]. Other studies reported on the quality and/or stability of decoctions, teas and infusions, aromatic waters, tinctures and mother tinctures, commercial instant teas of common herbal drugs and obtained important information about their preparation, content and shelf-lives.

Due to my long experience as a phytochemist and having at my disposal the excellent NMR facilities of Florence (CERM), one of the best equipped NMR groups in the world, I started a project using multidimensional NMR methods aimed at the analysis of complex spectra, such as those of plant extracts for their authentication, quality control and stability testing.

Studies were performed by the direct NMR analysis of complex plant mixtures, without purification or fractionation steps. Many matrices were used, such as SJW, ginkgo and ginseng extracts, an innovative supercritical carbon dioxide (CO_2) commercial extract of arnica, and samples of kava-kava herbal drugs and extracts [73-75].

Conventional methods used in herbal drug analysis (HPLC, HPTLC, GC, EC) can give a fingerprint of the markers or active constituents (and their percentage), but no information about the other metabolites of the extract, which can represent up to 95% of the total. This is true, especially in the unconventional extracts such as the innovative supercritical CO_2 extracts. The extract or the finely powdered herbal drug were directly dissolved in hexadeuterated dimethylsulfoxide and analyzed after filtration by NMR spectroscopy.

Spectral assignments of the constituents were carried out according to the data (chemical shifts and coupling constants) found in the literature and by means of 1D- and 2D-NMR spectra, which were found to be a valid alternative method to obtain a fingerprint for the assurance of content and stability and, as a consequence, safety and efficacy of extracts and herbal drugs. These studies represent a combination of fingerprint and semiquantitative analyses and although quantitation was only a minor aspect of these studies, the suitability of qHNMR to address questions of extract stability, as exemplified by the unstable *Hypericum* phloroglucinol derivatives, such as hyperforin, was clearly pointed out [56,73].

It was demonstrated that NMR experiments can provide a real and complete fingerprint of the extract, as required especially for innovative ones, to have a global vision and a "separation" of all constituents. All the molecules, as well as possible unknown or unexpected compounds can be detected. In addition, they can be used in the authentication of herbal drug material and to compare extracts manufactured with different processes and batch-to-batch analysis in the industry. NMR experiments can be considered a very simple, widely applicable and rapid analytical instrument, readily performed without pre-treatment, with tremendous versatility, not depending on the nature of the extract, inexpensive, perhaps with a lower precision than other methods but sufficient for pharmaceutical applications.

Another important line of research was dedicated to the application of biosensors. This important application was possible thanks to collaboration with a colleague and dear friend, Prof. Maria Minunni, and her chief, Prof. Marco Mascini, of the Department of Chemistry of the University of Florence. Our studies proved the applicability of sensors and biosensors for analysis in the search for new active constituents from plants, for the quality-control of HDs, HDPs and HMPs related not only to active or marker constituents but also to other substances, such as heavy metals and pesticides. Different biosensors (based on electrochemical transduction or on optical detection) were employed to evaluate the content of alkaloids of different extracts of *Chelidonium majus* L. (Papavaraceae), fractions obtained during "biosensor assay-guided" fractionation, pure constituents and on extracts submitted for stability testing. In addition, disposable sensors were used to detect heavy metals in samples of St. John's wort. A good correlation between the results obtained with the electrochemical devices and those from A.A.S. was observed [76,77].

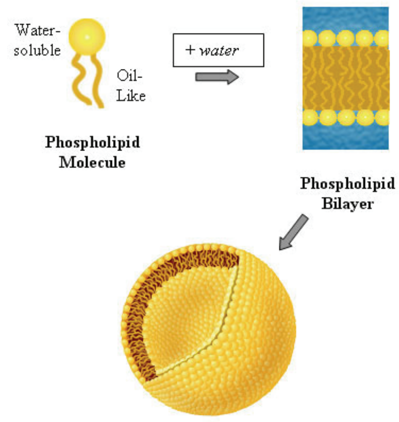

Figure 11: Formation of liposome, a typical pharmaceutical carrier, in aqueous solutions.

An important part of my research concerns the improvement of bioavailability and technological features of extracts, HDPs and HMPs by innovative methods (liposomes, ethosomes, supramolecular complexes, micelles) for which my thanks go also to Prof. Maria Camilla Bergonzi and many graduating and PhD students, among them Benedetta Isacchi, whom I consider to be my right arm. These preparations were analysed both by conventional methods (HPLC, DSC, UV, light scattering, dissolution tests) [78,80,81] and innovative ones, such as NMR (ROESY and DOSY) [79,82-84]. These studies were carried out on several supramolecular complexes between preparations of St. John's wort, kava-kava and cyclodextrins, micelles and liposomes (Figure 11).

I am really grateful to Prof. Gareth Morris of the University of Manchester (UK) for having applied and developed my initial idea of using diffusion-ordered spectroscopy (DOSY) methods for the analysis of micellar dispersions (octanoyl-6-O-ascorbic acid, SDS) and included molecules, such as artemisinin, curcumin, phloroglucinols, and anthocyanins. The investigations showed that DOSY experiments can yield both qualitative and quantitative information on the solubilization of nonpolar species by surfactants and the supramolecular complexes.

Finally, I should like to report some studies [85-89] with *Artemisia annua* L.. (Asteraceae, Figure 12) and artemisinin, a promising and potent antimalarial drug. These studies were carried out from the beginning in the framework of collaboration with a dear friend and colleague Prof. Luigi Messori of the Department of Analytical Chemistry of the University of Florence.

Figure 12: *Artemisia annua* L.

The interaction with hemin was first evaluated by UV/Vis spectrophotometry and HPLC/DAD/MS and the suitability of these simple methods for selection of new antimalarial compounds having similar properties was assessed. Furthermore the flavonoids isolated from *A. annua* were found to increase the rate of the reaction [85,86]. NMR studies of the supramolecular complex formed in the reaction were also carried out in collaboration with Prof. Paola Turano of the CERM [87]. Interesting results were also obtained using in the reaction hemoglobin instead of hemin [88]. Recent findings led to the discovery that green tea is also active against *Plasmodium*, and more importantly, its characteristic constituents, catechins, have a synergistic effect if administered with artemisinin [89]. Other studies on artemisinin and its extracts are also reported in this special issue.

This project still represents one of the most visible for the group, involving many other scientists and dear friends including Dr Carlo Severini and Dr Anna Rosa Sannella of the Istituto Superiore di Sanità, Rome, Italy, and Prof. Deniz Tasdemir of the School of Pharmacy, University of London (UK), and also attracting funds from the Sigma Tau (Pomezia, Rome, Italy), Ente Cassa di Risparmio di Firenze (Florence, Italy) and Toscana Life Sciences Foundation (Siena, Italy). Recently, a collaborative study with Prof. Carla Ghelardini and Prof. Nicoletta Galeotti of the Department of Preclinical and Clinical Pharmacology of the University of Florence has begun, with the aim of investigating antineuropathic activity of extracts, fractions and pure compounds from herbal drugs (a paper concerning these studies is reported in this issue). Finally, last but not the least, I would like to remember Prof. Vincieri's

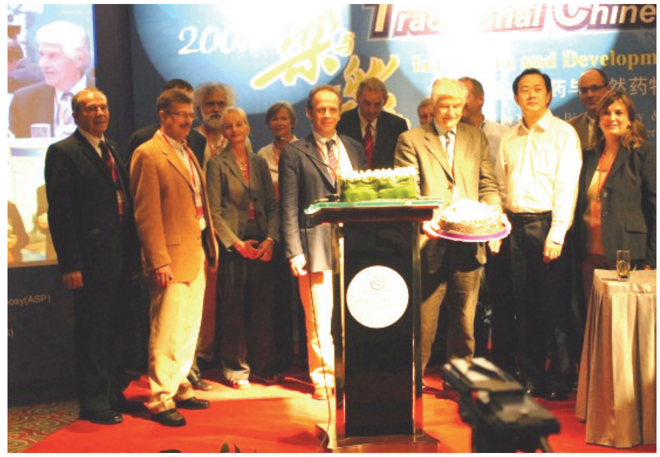

Figure 13: From the left side of the picture: Prof. K. Głowniak; Prof. T. Efferth, Prof. M. Hamburger, Prof. B.J. Baker, Prof. V. Butterweck, Prof. I. Merfort, Prof. L. Skaltsounis, Prof. R. Verpoorte, Prof. A. Nahrstedt, Prof. Prof. D. Guo, Prof. L. Pieters, Prof. A.R. Bilia and in the centre Prof. F.F. Vincieri on occasion of the celebration of his birthady during the 2008 Shangai International Conference on TCM and Natural Medicine.

collaboration with Prof. Anacleto Minghetti and Dr. Nicoletta (Nicky) Crespi Perellino, wonderful people and outstanding scientists who started their sincere friendship with Franco from the first time they met. They are also very active people and their expertise lies in the field of cell cultures [90] in particular.

The Vincieri research team is nowadays quite large, including three associate professors, three aggregate professors, four post-doctoral positions and about twenty students (including PhD, master's and graduate students) having two principal lines of research. The first is related to the development of specific methods of extraction, fractionation, isolation and characterization of potentially interesting secondary metabolites for pharmaceutical, alimentary and cosmetic fields. Other studies are directed towards the optimization of stability and technological and biopharmaceutical characteristics of herbal products, their extracts and commercial preparations.

I would like to conclude with a sentence to describe Prof Vincieri's profile: "an inspration to scientists, young and old, in all fields of research".

Figure 13 is a photo of Prof. Vincieri in Shangai for the International Conference on Traditional Chinese Medicine and Natural Medicine taken on his birthday, 12th October 2008. At that time he had a nice surprise and I hope this issue will be another even a greater one!

Happy birthday from all of us

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