Biodiversity as a Source of Medicines

Biodiversity as a Source of Medicines:

From Nature to the Market

Edited by

Valdir Cechinel Filho

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The present book is dedicated to:

My dear Family (Valdir Cechinel*, Amélia Copetti Cechinel*, Emílio Cecconi* and Bilmar Canarin Cecconi- * in memorian

My wife Lenita Cecconi Cechinel, my daughters Camile C. Cechinel Zanchett and Milene C. Cechinel, my genre Guilherme Zanchett and my grandchilds Samuel Cechinel Zanchett and Elisa Cechinel Zanchett.

My scientific fathers: Franco Delle Monache*, Rosendo A. Yunes and Mahabir P. Gupta*-* in memorian for all friendship, support, trust and teachings.

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CHAPTER 1

DRUGS FROM NATURAL SOURCES: HISTORY AND PERSPECTIVES

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Over time, humans have extensively used plants and other living sources as food and healing resources. At a scientific level there have been notable advances especially in the last three centuries and in recent times new investigative tools support human research activities. From the first observations of botanists and the analyzes of chemists, a large group of researchers, such as biologists, chemists, pharmacologists, doctors have improved their knowledge and, also supported by industries, are currently engaged in the research and evaluation of compounds of natural origin and their possible applications in different areas of human society. It is a contribution to the future of humanity and the sustainability of human progress, and bioactive compounds are expected to continue to play a useful role. Over time, humans have extensively used plants and other living sources as food and healing resources. At a scientific level there have been notable advances especially in the last three centuries and in recent times new investigative tools support human research activities. From the first observations of botanists and the analyzes of chemists, a large group of researchers, such as biologists, chemists, pharmacologists, doctors have improved their knowledge and, also supported by industries, are currently engaged in the research and evaluation of compounds of natural origin and their possible applications in different areas of human society. It is a contribution to the future of

humanity and the sustainability of human progress, and bioactive compounds are expected to continue to play a useful role.

Introduction

The evolution of human beings has been dependent and linked to the continuous increase in technological progress thanks to which they have become able to obtain nutrients and survive in different climates and environments, to establish living conditions that allow the survival of their progeny and to organize a complex society. The characteristic human talent in exploiting available natural resources has constituted fundamental milestones in the progress of human society. The observation and investigation of the complex system called "nature" has become "science", while the discoveries obtained from the investigation of nature have allowed the development of "technology"; technological products are not present in nature. Today, science and technology are playing a fundamental role in the development of human society, pushing towards new frontiers and perspectives.

The progress of human society is and will be linked to the discovery and subsequent application of biomolecules from the environment. Biomolecules are compounds derived from natural sources, such as plants, animals and microorganisms, that have biological activities (Baker et al., 2008) and are also known as secondary metabolites. The wide variability of organisms located in the sea, above and below the ground in different regions of the planet represents a high potential source of new biomolecules. Furthermore, these compounds present a great chemical diversity as the result of a process of evolution and adaptation to climate changes that have occurred over time through which the biosynthetic pathways have modified in response to various biotic and abiotic stresses (Hamann, 2006).

Biomolecules have been useful tools for humans and have found applications in various fields. For example, natural compounds were widely used for stringed musical instruments, and a mix of resins such as sandarac, rosin, elemi, amber, shellac, and additives or colorants such as dragon's blood, madder, and *Aloe vera* powders were the "secret" of violin makers in the past centuries (Kasprzok et al., 2019; Rombolà et al., 2023).

Natural compounds and biomolecules have also been used as traditional medicines, remedies, potions and oils; the use of these compounds occurred without any knowledge of chemistry and/or biology and the obtained results came from thousands of years of tests and experiments by humans (Kinghorn et al., 2011; Hicks, 2014).

The use of plants in traditional medicine is well known and documented (Krueger, 2005) and over time several medicines have been produced based

on folk knowledge from Ayurveda, Unani, Chinese, European, African, Central, South and North American cultures (Gurib-Fakim, 2006).

In more recent times, in advanced countries, the improvement of knowledge and their uses, more specifically pharmaceutical, has allowed the selection of the "active ingredient" of the biomolecule and the evaluation of its molecular mechanism(s) action; furthermore, the synthesis of bioactive molecules through chemical procedures developed in the laboratory allows for more precise investigations and tests, an accurate dosage of drug administration at a clinical level and consequently a wider diffusion and use of the products.

Currently throughout the world a large number of drugs derived from plants, and recently from other organisms, are used by humans, and in view of growing human needs and market prospects, research activities are constantly being improved to discover new biomolecules potentially useful for humans health [Gurib-Fakim, 2006; Mishra and Tiwari, 2011; Dias et al., 2012; Bernardini et al., 2017; Newman and Cragg, 2020). New perspectives are also coming from the use of plant biomolecules as environmental pharmaceutical products (Quattrucci et al., 2013), they are applied in the food industry and represent a new perspective for various sectors such as agrochemical and cosmetics (Hassan, 2012).

Traditional Plant Medicine: historical notes

For a very long time, in different regions of the planet and human cultures, plant biomolecules have been used as therapeutic agents and can be considered some branches of Traditional Medicine.

Traditional Chinese Medicine

This is a very ancient system of medicine, believed to be more than 5000 years old, and is based on two separate theories about the natural laws that govern good health and longevity, namely *yin* and *yang*, and the five elements (wu xing) of the *earth, metal, water, wood* and *fire* relating to the main organs of the body. Traditional Chinese Medicine has been documented for thousands of years (Unschuld, 2016) and is collected largely in the Chinese Materia Media (1100 BC) (Wu She Er Bing Fang, containing 52 prescriptions), in the Shannong Herbal (around 100 BC, containing 365 medicines) and the Tang Herbal (659 BC, containing 850 medicines) (Cragg & Newman, 2005).

The Modern Day Encyclopedia of Chinese materia medica published in 1977 is the most complete reference on Chinese herbal prescriptions and

lists approximately 6000 medicines, 4800 of which are of plant origin. Chinese herbs are usually administered in fixed mixtures containing a maximum of 20 herbs (Chang and But, 1986). In recent times, Traditional Chinese Medicine has spread to Western countries and new investigations have been conducted to evaluate the effects of healing preparations, the active ingredients contained in them and their mechanisms of action.

Ayurvedic medicine (traditional Indian medicine)

The term Ayurveda derives from the Sanskrit words "*Ayar*" (Life) and "*Veda*" (Science or Knowledge) and is one of the oldest medicinal practices; even currently it is widely used and applied in India. Ayurvedic medicine is considered a holistic approach to the health of the human body and mind. The philosophical principles and use of herbs as medicinal plants suggested by this approach are contained in thousands of poetic hymns included in a text called the Rig Veda, supposedly written about 2000 years BC. although documentation on the Ayurvedic system dates back to 1st millennium BC (Patwardhan 2005). Ayurvedic medicine had influence on Greek and Middle Eastern medicinal traditions (Samy et al., 2008) and is also popular in Western countries. In Russia it was introduced after the Chernobyl disaster (Ragozin, 2016).

Traditional Arab (Unami-Tibb) and North African medicine

Archaeological studies have informed that in the Arab area and North Africa biomolecules have been used as therapeutic agents for a long time. The first reports of the use of biomolecules as medicines were depicted on cuneiform clay tablets from Mesopotamia (2600 BC), which reported a medicinal system composed of approximately 1000 plant-derived medicines (Cragg & Newman, 2005). Furthermore, the Code of Hammurabi (ca. 1700 BC), in addition to the laws, also reported a list of medicinal herbs. From before 3000 BC the Egyptians wrote their medical and pharmaceutical knowledge on papyrus and in tomb frescoes. Interesting information was recorded in the Ebers Papyrus (named after Georges Ebers who discovered the papyrus in 1872) written around 1550 BC, the 9th year of the reign of Amenhotep 1, which contains over 700 drugs of plant origin (Borchardt, 2002; Schedler, 2006; Cragg and Newman, 2013).

The Arabs retained much of the Greco-Roman medicinal knowledge and improved and expanded it to include their own resources. They also contributed to spreading Chinese and Indian cultures in the use of herbs to the West. In the 8th century, pharmacies arose in the Arab regions and the

famous Persian pharmacist (also doctor, philosopher and poet) Avicenna (Abù'Alì al Husayn ibn Sinà) wrote the "*Canon medicinae*", a fundamental treatise on Medicine which included elements from other cultures medicines and provided information for a distinct system of Islamic healing. This system is now known as Unani-Tibb (Jabin, 2011).

Traditional European medicine

In the ancient Western world, the Greeks and later the Romans contributed extensively to the rational use of herbal medicines and the healing system, and Hippocrates (460-377 BC) and Aristotle (384-322 BC) played vital roles; the roots of Greek healing culture came from the ancient healing cultures of India (partly through Egyptian healing culture). The Greek Theophrastus (around 100 BC) wrote about medicinal herbs; the Roman Pliny the Elder (1st century AD) reports news on the collection, conservation and use of medicinal herbs (Schedler, 2006). The Greek physician Dioscorides (1st century AD) reported information on the collection, preservation and use of medicinal herbs (Atanasov et al., 2021). Dioscorides, who travelled with the Roman armies throughout the Empire, first introduced the term "botanike" into writings on plants. He was the author of the famous book "De Materia Medica", in which 813 medicinal herbs were reported. De Materia Medica is one of the oldest European herbal treatises and has been the reference source in Europe for more than 1000 years. Galen (130-200 AD), a Greek scholar of pharmaceutical and medical sciences active in Rome, published 30 books on these topics and is still known for his prescriptions and formulas used to prepare medicines, sometimes containing dozens of ingredients called "galenic".

In the Late Middle Ages in Central Europe, medicinal plants were cultivated according to a standardized scheme in monasteries, and in Germany the Benedictine abbess Hildegard of Bingen (1098-1179) is still remembered as a famous healer and founder of natural-historical science. In Italy, a particular contribution to the development of a culture of healing came from the studies of Pietro Hyspano (then Pope John XXI, 1205-1277) with his book entitled "Thesaurus Pauperum". In the 15th and 16th centuries in Europe occurred the invention of letterpress printing by Johannes Gutenberg made it possible to print and distribute herbal books such as The Herbarium of Mainz (Herbarius Moguntinus 1484), the German Herbarium (1484), the Herbarium Vivae Eicones (Otto Brunfels 1530), the Kreütter Buch by Hieronymus Bock (1546), the De History Stirpium by Leonhart Fuchs (1542) the treatise "Pedanii Dioscoridis Anazarbei De medica

materia libri sex" by Pier Andrea Mattioli (1546) the book "Herbario Nuovo" by the naturalist Castore Durante (1585)

Currently, phytotherapy is widespread in Europe.

Traditional African medicine

It is well known that Africa is the place of origin of man and consequently traditional African medicine is probably the oldest of all medical systems. Traditional African medicine involves both body and mind and is a mix of herbalism and spirituality. Unfortunately, it remains poorly recorded. Intense social processes are occurring in Africa, as well as deforestation and habitat loss of some medicinal plants. To preserve knowledge of the traditional uses of medicinal plants, documentation must be thorough and documented. African botanists and chemists offer their valuable contribution (Ogunlesi, 2008).

Traditional medicine in Central and South America

The regions of Central and South America are characterized by great plant biodiversity and over time various healing cultures have developed, which are still little known and not adequately documented on a scientific level. As demonstrated by archaeological studies on Chilean mummies dated 2000 BC. to 1500 BC, chemistry of ancient hair samples showed the cocaine metabolite benzoylecgonine (Baez et al., 2000), and nicotine was found in hair samples from Nubian burial sites of children and adults (Balabanova et al., 1996). The use of hallucinogenic substances derived from the toasted seeds of the leguminous plant *Anadenanthera peregrina* was also widespread. Many compounds from plants in Central and South America have been characterized and applied as active ingredients for pharmaceutical products in several countries. In recent times the use of Aloe plant leaf extracts against cancer has spread from South America to Europe, unfortunately without appreciable results.

Traditional medicine in North America

As in other ancient cultures, in the United States and Canada the indigenous healer, known as the Shaman, had a physical and spiritual approach to illness. Herbal cures from native practices formed the basis of the United States Pharmacopoeia; recently, herbs have become popular in the United States and Canada primarily as nutritional and pharmaceutical supplements rather than as medicines. Depending on the multicultural content of the US

populations, in such a country all the healing cultures mentioned are widespread and find application.

Plants as a source of bioactive molecules

All living organisms have a metabolism, called primary metabolism, which produces fundamental molecules to ensure living conditions and reproduction. Furthermore, plant organisms are characterized by a secondary metabolism, responsible for the production of molecules, called secondary metabolites, whose biological role remained unclear for a long time. It is now established that these compounds play fundamental roles in defense, communication and reproduction, act as intermediaries between plants and other living components of the environment, have long-term effects on plant growth and allow them to survive even in the presence of environmental stress. (Kurepin et al., 2017). Such compounds have also proven to be a valuable and almost inexhaustible source of molecules for developing new drugs and active ingredients for pharmaceutical products, and since ancient times it has been documented that natural compounds play a fundamental role in the treatment or prevention of various diseases (Yuan et al., 2016). Although the use of natural products in the formulation of herbal pharmaceutical preparations was already a practice in human culture hundreds and thousands of years ago, their study and application as bioactive compounds only began in the 19th century (Veeresham, 2012; Bernardini et al., 2017).

The biological activity that plants have demonstrated to possess, and their application in the clinical-pharmaceutical field, depend to a large extent on their chemical constituents: they are capable of biosynthesizing a wide range of molecules, called secondary metabolites, which represent a subset of the plant metabolome (Wang et al., 2019). Approximately 100,000 different secondary metabolites have been identified in the plant kingdom, classified into three main groups based on their biosynthetic pathway: nitrogen-containing compounds (cyanogenic glycosides, alkaloids and glucosinolates), phenolic compounds (flavonoids and phenylpropanoids) and terpenes (isoprenoids) (Fang et al., 2011).

Historically important Bioactive Molecules for Pharmaceuticals

Current pharmaceutical products originate from traditional medicine. For a long time and everywhere, plants were used only as “magic” remedies without any knowledge of the molecules that compose them. Then, a greater capacity for observation and evaluation made it possible to understand that

living matter is made up of molecules and that it was possible to proceed with the identification and isolation of the molecules.

An example of a drug derived from a bioactive molecule is the anti-inflammatory agent acetylsalicylic acid, derived from salicin, present in the bark of the willow *Salix alba* L. (Der Marderosia and Beutler, 2002), then produced by chemical synthesis in 1853. The use of Willow bark as a healing plant has very ancient origins because the Egyptians already used willow bark infusions to treat fever. In the 18th century, digitoxin from *Digitalis purpurea* L. (digitalis) was found to be a cardiotonic glycoside capable of improving cardiac conduction and improving the strength of cardiac contractility (Der Manderosian and Beutler, 2002). Quina tree extract is another example of a plant active ingredient standardized as a pharmaceutical. It was used in Peru by the indigenous people against malarial fever induced by four species of the Plasmodium parasite, namely *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*. In the 17th century the cinchona tree was imported into Europe and used as a powder with the name "Jesuit Powder" and at the beginning of the 19th century an active ingredient consisting of an alkaloid called quinine was isolated (Eiden, 1999; Felisati, 2000). Another example is the alkaloid morphine isolated in 1803 from *Papaverum sonniferum* L (opium poppy) and currently widely used as a commercial drug. Because it tends to cause sleep, it was originally called morphine in honour of Morpheus, the Greek god of dreams (Fisher, 2009; Mosher, 2013).

Since 1870 crude morphine was converted in diacetylmorphine to get heroin and readily converted to codeine. It is interesting to note, thus confirming the origin of pharmaceuticals from traditional medicine, that poppy extracts were already used by Sumerians and ancient Greeks, and Arabs (Der Manderosian and Beutler, 2002). After morphine isolation many bioactive molecules, mostly alkaloids (e.g. atropine, brucine, caffeine, capsaicin, cocaine, codeine, colchicine, febrifuge, nicotine, quinine, strychnine) were isolated and purified from plants x (Schedler, 2006; Dias et al., 2012; Veeresham, 2012).

Currently, thanks to continuous progress in the fields of chemistry and biology, it is possible to proceed with a real "disassembly" of the molecular composition of plants and isolate, characterize and test individual bioactive molecules. Many substances of plant origin are also used as a basis for the development of drugs with advantageous characteristics: increased activity, reduced toxicity, improved pharmacokinetics and pharmacodynamics. However, since extraction, purification and/or (when possible) semisynthesis remain inefficient in some cases, inexpensive processes are underway to obtain these molecules, such as the use of bacteria or other engineered organisms (Courdavault et al., 2020).

Over time, some plant bioactive molecules have been used as anti-tumor drugs:

- Colchicine, a compound that promotes microtubule depolymerization (Ghawanmeh et al., 2017), was originally extracted from plants of the genus Colchicum (*C. autumnale*) and isolated in 1820 by two French chemists, Pelletier and Caventou. Colchicine binds to tubulin, forming tubulin-colchicine complexes and blocking the polymerization of microtubules, cytoskeletal elements that play a fundamental role in various cellular processes. After its use as an anticancer compound, it has more recently been used to treat various diseases such as rheumatism (Slobodnick et al., 2017) and gout (Dalbeth et al., 2014).
- Paclitaxel (Taxol®) is a popular drug used to treat lung, breast and ovarian cancer. Taxol and its derivatives work by blocking microtubule depolymerization and mitosis and inducing apoptosis (Mamadalieva and Mamedov, 2020). The first isolation of Taxol® from the bark of *Taxus brevifolia* was performed by Monroe Wall and Mansukh Wani at the United States Department of Agriculture (USDA) and dates to 1967 (Cragg, 1998); the first FDA approval dates back to 1992 (Cseke et al., 2006). Due to the low extraction yield from the bark and the high demand for the drug, paclitaxel is currently produced synthetically (Dewick, 2009). Since the process is demanding and expensive, a semi-synthetic production method was established from taxans collected from *T. brevifolia* leaves.
- Vinblastine and derivatives (Vinca alkaloids), alkaloids extracted from *Catharanthus roseus* G. and act through binding to β-tubulin, which leads to an inhibition of microtubule polymerization, blocking the cell cycle in M phase (Srivastava et al., 2005).
- Irinotecan, a camptothecin extracted from the bark of *Camptotheca acuminata*, is a prodrug activated by metabolism in its active form SN-38. It inhibits topoisomerase 1, causing apoptosis and is used to treat advanced and/or metastatic gastric, pancreatic, and colon solid tissue tumours in combination with other drugs (Bailly, 2019).
- Podophyllotoxin extracted from *Podophyllum peltatum*, and especially its semi-synthetic form derivatives such as the lignans etoposide and teniposide, act by destabilizing microtubules and by inhibiting topoisomerase 2. They are used in the treatment of lymphomas, leukemias, neuroblastoma, testicular, lung and ovarian cancer (Courdavault et al., 2020).

Many other active compounds have been isolated and characterized mostly at a chemical level, isolated from natural products and with growing interest, until the advent of synthesis techniques which were believed to be able to completely replace the natural origin. However, since research in this direction has not produced the expected levels of efficacy and productivity, today greater attention is being paid to natural products in the search for new drugs in combination with new technologies (Zhu et al., 2012; Bernardini et al., 2017).

Natural compounds from fungi

Important compounds with therapeutic activities have been collected from non-plant organisms, such as fungi. Generally, they can be microscopic to macroscopic and include unicellular organisms such as yeasts and multicellular organisms such as filamentous fungi. Since ancient times, the so-called mushrooms, namely Basidiomycota, have been used as food and for medicinal purposes, especially in traditional Asian medicine. Metabolites of Basidiomycota (or higher fungi) demonstrate verified pharmacological activity in major diseases such as chronic inflammation, diabetes, oxidation-associated pathologies, infections (HIV, bacteria, other fungi), immune system disorders and cancer (Poucheret et al., 2006).

More than 270 medicinal fungi are reported in Traditional Chinese medicine for their preventive and/or curative effects. Four species are very popular in Asian medical care: Shiitake (*Lentinula edodes*), Reishi or Mannentake (*Ganoderma lucidum*), Maitake (*Grifola frondosa)* and Enokitake (*Flammulina velutipes*). Lion's Mane (*Hericium erinaceus),* Cordyceps *(Ophiocordyceps sinensis),* and Turkey Tail *(Trametes versicolor)* are also considered new-generation foods and are of growing interest to consumers, classified as functional foods thanks to their high content of biologically active compounds, including fibers, triterpenes, phenolic compounds, and sterols (Łysakowska et al., 2023). Known pharmacological activities of higher fungi, due to their Basidiomycota biologically active metabolites (BAM), are mainly: antioxidant activity, anti-inflammatory effects, immunostimulant activity, antitumoral activity, antimicrobial activity and activity on metabolic syndrome and related diseases, such as diabetes and hypertension (Poucheret et al., 2006).

Regarding the plant kingdom, higher fungi are a natural source of potent bioactive antioxidant metabolites, such as phenolic compounds but also numerous secondary metabolites (Yang et al., 2002). Furthermore, mushrooms not only demonstrate direct antioxidant capabilities but are also able to stimulate the host's anti-radical defense: the antioxidant enzymes of subjects

who consume mushrooms are specifically regulated (SOD, GPx, catalase) (Park et al., 2001). *Pleurotus ostreatus* and *Agrocybe aegerita* are two of the most antioxidant higher mushroom species: they contain many phenolics and basic antioxidant compounds (ascorbic acid, tocopherol, β-carotene) (Yang et al., 2002; Lo and Cheung, 2005).

Anti-inflammatory effects are shown by a lot of compounds isolated from various fungi: fatty acids and sterols extracts of *G. frondosa* and *A. aegerita* that inhibit lipidic mediators of inflammation such as cyclooxygenases (Hong et al., 2004; Zhang et al., 2002, 2003) or ethanolic extract of *Lentinus crinitus* and *Agaricus subrufescens* that lowers cytokines level (NF-κB, IFN-γ and TNF-α). Glucans and polysaccharides of *Trametes gibbosa, Cordyceps militaris, P. floridanus* and *G. lucidum* have shown anti-inflammatory potential on cell models as well as on animal models for both acute and chronic inflammation (Jose et al., 2004; Lakshmi et al., 2003).

Other species of microscopic fungi of the Ascomycota are also a source of interesting molecules. Trichodimerol, a natural molecule first isolated from *Trichoderma longibraciatum*, was found to reduce the production of NO, ROS, and interleukin (IL)-6 (Huo et al., 2022). These are just a few examples of antioxidant and anti-inflammatory activities by molecules present in mushrooms.

Due to their medicinal and functional properties, superior mushrooms are increasingly used in human nutrition, also adding dried and powdered mushrooms (1 – 15%) to foods (Łysakowska et al, 2023).Indeed *Auricularia auricula-judae, G. lucidum, G. frondosa, Lyophyllum decastes* and *Cordyceps sinensis* can reduce hyperlipidemia and hypercholesterolemia, or regulate triglyceride levels, with positive effects on diabetes and hypertension (Bobek et al., 1991; Gunde-Cimerman and Plemenitas, 2001; Kamiya et al., 1969; Kubo and Nanba, 1997; Ukawa et al., 2001).

Many higher mushrooms (*Agrocybe cylindracea, C. sinensis, Tremella aurantia, Agaricus bisporu*s, etc.) show a hypoglycemic effect, while some others (*G. lucidum, G. frondosa, Tricholoma mongolicum and Macrocybe gigantea*) have the ability to reduce blood pressure high and related pathologies such as angina pectoris and atherosclerosis (Kabir et al.,1988). Mushrooms can therefore positively regulate events that can lead to cardiovascular disease, diabetes, obesity and even neurodegenerative diseases (Tsukamoto et al., 2003).

Several species of fungi demonstrate immunostimulatory activity, for example *Lentinula edodes* produces Lentinan, a compound that increases the host's immune system and defense against external viral infections such as HIV and AIDS (Poucheret et al., 2006). *Trametes versicolor*, *Grifola frondosa* and *Ganoderma spp* are also powerful immunomodulatory fungi:

through their metabolites (PSP and PSK, glucans, LZ-8 and triterpenoids), they induce the production of cytokines (IL), interferon (IFN), tumor necrosis factor (TNF) and stimulate the activity of cytotoxic T cells, Natural Killer cells (NK cells), B and T lymphocytes and macrophages. These effects translate into a generic improvement of the humoral and cellular immune response of the host (Kino et al., 1989; Ng, 1998; Cui and Chisti, 2003; Lin, 2005; Cao and Lin, 2006).

Nowadays, more than 200 species of higher fungi exhibit antibacterial activities. Many species also possess antifungal, antiparasitic and antiviral properties (Anke and Sterner, 1991). Some even show combined biological properties such as *Agrocybe molesta* (antibacterial and antifungal) and *Flammulina velutipes* (antifungal and antiviral) (Poucheret et al., 2006).

Antitumor activity is another significant therapeutic activity associated with mushrooms, it concerns over 50 higher fungi on various tumors and types of tumor processes (Cochran, 1978; Kidd, 2000; Tomasi et al., 2004). Previous studies have shown that Basidiomycota metabolites are a potential source of anticancer agents, particularly polysaccharides, often β-glucans with various types of branching (Ebina et al., 2004; Fuji et al. 1978; Kerrigan, 2005; Lu, 1995; Mizuno et al., 1995 Yoshioka et al., 1985).

Not only polysaccharides show antitumor activity; other examples of useful metabolites are:

- Clitocypin, from *Clitocybe nebularis,* a cystein protease inhibitor studied against rheumatoid arthritis (Brzin et al., 2000).
- Illudin S, from *Omphalotus illudens,* a very strong antibiotic directed against *Staphylococcus aureus* (Lehmann et al., 2003) but also demonstrate anticancer properties against solid tumors working as a DNA-alkylating agent, selective for tumoral cells (Leggas et al., 2002; Yeo et al., 2006).
- Putrescine-1,4-dicinnamide, from *Pholiota spumosa,* useful against human prostate cancers (Russo et al., 2006).

Clearly microscopic fungal species have also been studied and the presence of biological activities within various divisions, families and species of the fungal kingdom is now widely accepted.

A classic example is penicillin, a bioactive molecule produced by the fungus *Penicillium notatum* and discovered by Fleming in 1929 (Mann, 1994); subsequently Chain and Florey carried out *in vivo* testing which allowed numerous human lives to be saved all over the world. The three scientists were awarded the Nobel Prize for Physiology or Medicine in 1945 (Stamets, 2002). The discovery of penicillin opened up new possibilities in

drug discovery research, and new antibiotics such as streptomycin, gentamicin, tetracycline, and others were discovered (Buss and Waigh, 1995; Williams, 1999); furthermore, the production and marketing of synthetic antibiotics began (Brockmann, 1970; Wainwright, 1990; Mann 1999; Fay, 2005).

Other examples of active compound is the cyclosporine, produced by *Tolypocladium inflatum*, (Iram and Anjum, 2015) and used as an anti-inflammatory and immunosuppressant drug, as well as the lovastatin produced by *Aspergillus terreus*, a compoud able to reduce the blood cholesterol (Karwehl and Stadler, 2016). The metabolites of Ascomycota mushrooms also have the same variety of effects as BAM. Endophytic and marine fungi also show many secondary metabolites: for example, in recent decades, 237 new diterpenes have been isolated from 47 strains of marine fungi belonging to 15 genera (Qiu et al., 2022) and 232 new bioactive metabolites from the genus Marine-derived Aspergillus (Wang and Ding, 2021).

Mushrooms are also useful for pest control in agriculture: microscopic entomopathogenic fungi can kill the host through mechanical damage, malnutrition and toxins, much depends on the type of mushroom and its mechanism of action (Khan et al., 2012). The most widely used species worldwide for pest control include *Metarhizium anisopliae* (33.9%); *Beauveria bassiana* (33.9%); *Isaria fumosorosea* (5.8%) and *Beauveria brongniartii* (4.1%) (Faria and Wraight, 2007). They produce various toxins that affect the host, as amatoxins from the Amanita genus, destruxins from *Metarhizium anisopliae*, beauvericin and bassianolide from *Beauveria bassiana*.

Macroscopic fungi also exhibit insecticidal activity, thanks to secondary metabolites and other compounds, especially proteins such as hemolysin, lectin, serpin proteins, aegerolysin, ostreolysin A6, pleurotolysin A2 and erysin of various kinds. In total, more than 150 fungi with insecticidal activity have been evaluated between 1876 and 2021 (Castañeda-Ramírez et al., 2022).

Although more and more secondary metabolites have been obtained from fungi, exploitation of their biosynthetic potential is far from sufficient and many of their biological activities have yet to be discovered.

Other sources of natural compounds

Continuous improvement of techniques has offered new possibilities of investigation and opened new perspectives to expand the studies on the sources of biomolecules; consequently, many living organisms are currently studied as possible sources of bioactive molecules. Lichens, symbiotic organisms resulting from the association of an autotrophic organism, a cyanobacterium or an alga, and a heterotrophic one, a fungus, have been studied

in depth in more recent times for their content of biomolecules and the prospects for pharmaceutical applications (Saklani and Upreti, 1992; Behera et al., 2003; Munzi et al., 2014; Xu et al., 2016; Studzińska-Sroka et al., 2021; Tripathi et al., 2022). Furthermore, more recently researchers have focused their scientific interest on marine organisms, opening up prospects for new applications in the pharmaceutical field (Simon et al., 2010; Riccio et al., 2020; Mohamed et al., 2024). Investigations and tests on the biological activities of oleates (Bernini et al., 2017; Caroleo et al., 2021; Laghezza Masci et al., 2022), hydrolates (Rajeswara, 2012; Verma et al., 2012; Giacometti et al., 2018; Ovidi et., 2021; Ovidi et al., 2022) and essential oils and volatiles (Ramos et al., 2011; Nieto, 2017; Mutlu-Ingok et al., 2020; Garzoli et al., 2021; Iannone et al., 2022) open up also to new possibilities for new biomolecules.

New Findings and Perspectives for Research Investigations on Natural Substances

Over time, dependent on the lack of knowledge of the chemical composition, the use of natural substances and bioactive molecules has moved from an "empirical" use of plants to a more precise application of their derivatives and biomolecules. Supported by continuous technological progress, research investigations have offered more precise information on the nature of active biomolecules, their mechanisms of action, and more correct dosage and use in order to reduce possible unwanted diseases. Furthermore, more recent investigations conducted on other living organisms have expanded the range of "sources" of these compounds and have opened new perspectives in the search for biomolecules to be applied in various fields of human activities and in particular in the pharmaceutical field. It is interesting to note that prospects for new molecules of pharmaceutical interest could also derive from the study of specific plants used by animals in self-medicating diets. For example, plants ingested by our "distant cousins" such as wild macaques of Japan and wild chimpanzees of Uganda have shown, respectively, antiprotozoan and anthelmintic properties (Trasdemir et al., 2020) and potent antimicrobial activities (Freymann et al., 2024).

Humanity needs new drugs to fight bacteria and possible upcoming pandemics, and new drugs are needed to fight cancer and many other pathologies. Furthermore, probably dependent on new lifestyles (such as, for example, changes in diet and in the quality of nutrition, contamination and pollution of the environment and food, planning and use of different rhythms of life, increased competition in many human activities and, consequently, increased stress), new pathologies defined as "rare" are occurring

in different regions of the world. Pathologies that need to be investigated and combated using new pharmaceutical tools.

For a long time, pharmaceutical properties of plant extracts took place through *in vivo* tests carried out by evaluating the level of affection directly on humans; currently, thanks to the continuous progress of knowledge and the contribution of public and private research activities in various countries, science and technologies have offered new tools, new investigation strategies and new possibilities for reducing the effects of pathologies.

Optical and electron microscopy investigations

Thanks to human ingenuity, the invention and development of the lens and new derivative instruments, such as the telescope and the microscope, have opened up new perspectives in many fields. With the telescope, the scientific study of the sky took hold and modern astronomy began; through the microscope a new discipline started, microscopy, and man learned the smallest components that constitute living organisms and in particular it was possible to understand the cellular components of the human body, the cellular anatomy of animals and insects, the peculiarities of plants, the structure of algae and fungi. Microscopy has become an important tool for scientists, and the technology has been able to improve the quality and performance of the instruments. Currently on the market there is a wide range of optical microscopes such as fluorescence and confocal microscopes integrated by specially dedicated computerized machines, and transmission and scanning electron microscopes supported by specific computerized machines. New techniques for fixing and treating the investigated materials have also been developed, improving the level of definition of the observed ultrastructure; further, very useful information has been provided by the use of antibodies markers of specific proteins. These new instruments and technologies have offered significant contributions to the study of cellular structures and subcellular components influenced by bioactive molecules and have opened up multidisciplinary research approaches for the investigation and characterization of natural compounds.

Optical and electron microscopic investigations were conducted to study the level of affection of *Aloe arborescens* leaf extracts (Ceccarelli et al., 2012). An interesting article confirming the importance of microscopy in visualizing the level of effect of bioactive molecules on the in vitro system was reported by Donoso-Fierro et al. (2014), who tested yatein, a compound isolated from *Astrocedus chilensis*, on murine myeloma cells. A combination of optical and electron microscopic observations helped to better understand the level of influence of yatein on the microtubular apparatus and

at the ultrastructural level of organelles and membranes. Microtubules were also considered as a cellular target to test the level of affection of a lipid fraction of *Scutellaria ramosissima* (Mamadalieva et al., 2014) and to evaluate the affection of Salvia leaf extracts (Ovidi et al., 2018). Scanning electron microscope observations supported the study on the effects of *Papaver rhoeas* ovule extracts on human leukemic cells (Ovidi et al., 2020).

The imaging technique of confocal microscopy played a pivotal role in drug discovery and development from natural sources. Since its inception in the late 20th century, confocal microscopy has transformed the way researchers visualize and analyze biological samples, offering high-resolution images and three-dimensional reconstructions. In the context of drug discovery, confocal microscopy has been instrumental in studying the interactions between drugs derived from natural sources and their molecular targets within cells. By providing detailed insights into subcellular localization, drug uptake, and intracellular trafficking, confocal microscopy aids in elucidating the mechanisms of action of natural compounds. (Pedro and Rudewicz, 2020).

One of the key advantages of confocal microscopy is its ability to perform live-cell imaging, enabling researchers to monitor dynamic processes in real time. This capability has been invaluable in assessing the effects of natural products on cellular dynamics, such as cell proliferation, apoptosis, and signaling pathways. Furthermore, confocal microscopy allows for the visualization of cellular structures with high precision, facilitating the identification of morphological changes induced by natural compounds. This information is crucial for evaluating the efficacy and toxicity profiles of potential drug candidates derived from natural sources. (Atanasov et al., 2021).

The integration of confocal microscopy with advanced imaging techniques, such as fluorescence resonance energy transfer (FRET) and fluorescence lifetime imaging microscopy (FLIM), has further expanded its applications in drug discovery. These techniques provide additional insights into molecular interactions and signaling events, enhancing the understanding of drug mechanisms and cellular responses. The historical evolution and current perspectives of confocal microscopy underscore its significance in exploring the pharmacological properties of drugs derived from natural sources. By offering detailed visualization, dynamic imaging capabilities, and molecular insights, confocal microscopy continues to drive innovations in pharmaceutical research (Ishikawa-Ankerhold, et al., 2012).

The use of the microscopy research approach has proven to be very useful for studying and evaluating the effects of bioactive molecules at a microscopic and ultrastructural level. It is certainly a very powerful tool for a

multidisciplinary approach to better characterize bioactive molecules and plan their use in the pharmaceutical field.

2d and 3d cell cultures: new perspectives for in vitro investigation of drug activities

The development of innovative *in vitro* models for studying cell biology and physiology is of great importance in the fields of medical research, drug discovery, toxicity testing, as well as for the emerging fields of tissue engineering and regenerative medicine (Achilli et al., 2012).

In vitro cell models are in fact the basis for studying the effects of substances of pharmacological interest. The growth of monolayer cell lines on media such as common cell culture media (flasks or Petri dishes) has severe limitations on the responses of cells to the different substances under study (Achilli et al., 2012). In 2D techniques, cell-plastic interactions prevail rather than the *cell to cell* and *cell to extracellular matrix* (ECM) interactions that form the fundamental basis for proper cell function. Furthermore, the plastic support is an unnaturally rigid substrate compared to the mechanical and softer environment that cells experience *in vivo*, which is known to affect cell function (Kleinman et al., 2003)

In recent studies aimed at developing new drugs, the problem most frequently encountered by researchers is that promising preclinical activity often does not transfer to the clinical situation when the drug is administered to the patient (Colotta et al., 2008). It has emerged that 2D study models, due to their characteristics, tend to overestimate the effects of chemotherapeutic agents administered to them compared to their counterparts in three dimensions (Kunz-Schughart et al., 2004).

Of the substances that proved promising and active in 2D models in clinical trials, only 5% showed *in vivo* efficacy equal or like that *in vitro*. This is a clear indication of the inadequacy of the two-dimensional model for drug testing (Hutchinson and Kirk, 2011).

In the absence of an adhesion surface or matrix, the cells will tend to spontaneously aggregate forming three-dimensional microtissues (Duguay et al., 2003) and to simulate the *in vivo* conditions more efficiently and recreate the microenvironment in which the cells are found, 3D cell models have been developed *in vitro* (Chaicharoenaudomrung et al., 2019).

In this type of cell culture, on liquid medium or on a matrix, the tendency of cells to form aggregates and spheroids is exploited. Although a spheroid cannot perfectly mimic a human tissue, with all its cell-cell and cell-matrix interactions, it comes close to the point where cells assume a morphology and gene expression comparable to what they would have within the organism of

origin. Furthermore, a 3D model can be kept in culture for longer because it does not have the limitation of spatial confluence achieved by cells on a 2D monolayer (Chaicharoenaudomrung et al., 2019).

Developing in 3D, the spheroidal cell aggregate has factors that influence the effect of the drug being tested. Specifically, within the spheroidal structure, various cellular stages are established due to nutrients and oxygen, and different cellular condition can be detected: proliferating cells, quiescent cells, apoptotic cells, hypoxic and necrotic cells (Kim et al., 2005).

For the formation and development of these cell aggregates, hydrogels or culture media enriched with substances designed to mimic a natural extracellular matrix are used. These include hyaluronic acid, collagen, gelatin, fibrin, alginate, and agarose (Dean et al., 2007).

In addition, inert and biocompatible materials can be used to create solid scaffolds with different porosity, permeability, and mechanical properties. Collagen, gelatin, silk, chitosan, and alginate are produced for this type of culture. This is followed by a drying phase that can be performed using different techniques, of which freeze-drying is the simplest and most effective (Wu et al., 2010).

As the advantages of 3D culture systems have been widely recognized, many studies have been conducted focusing on the development and optimization of cell culture technologies in 3D, which have led to the consolidation of six main culture techniques. These include liquid overlay culture, hanging drop, hydrogel embedding, spinner flask bioreactor, scaffold, 3D bioprinting (Chaicharoenaudomrung et al., 2019).

Applying this type of cell culture in the clinical-pharmaceutical sector, as in the studies concerning the response to drug administration, these treatments are not immediately absorbed by all cells, but in the inner zones, where there is a hypoxic condition of the tumor mass, different effects are generated that lead to increased and progressive drug resistance on the part of these cells (Chaicharoenaudomrung et al., 2019).

Flow cytometry investigations

Flow cytometry is a technique that has found various applications in biology and in the discovery and development of drugs from natural sources, evolved into an indispensable tool for pharmaceutical research. One significant aspect of flow cytometry in drug discovery is its ability to analyze individual cells within a heterogeneous population, allowing for precise characterization of drug effects on specific cell types. This capability has been instrumental in screening natural compounds for their pharmacological activities and mechanisms of action. (Ullas et al., 2024).

Flow cytometry enables high-throughput screening of large libraries of compounds, accelerating the identification of bioactive molecules from natural sources. By analyzing cellular responses to these compounds, researchers can decipher their therapeutic potential and optimize drug development processes. The integration of flow cytometry with other techniques, such as high-content screening and multiparametric analysis, has improved its utility in drug discovery from natural products. This multidimensional approach provides a comprehensive view of the biological activities of natural compounds, facilitating the identification of guide molecules for drug development. (Ding and Baker, 2021).

The historical evolution and current perspectives of flow cytometry underscore its significance in the discovery and development of drugs from natural sources. By offering high-throughput analysis, single-cell resolution, and multi-parametric data acquisition, flow cytometry continues to catalyze advancements in pharmaceutical research (Aru and Yanikkaya Demirel, 2024).

In vitro tests to evaluate drug-induced resistance

Bioactive molecules are currently used as pharmaceutical products against cancer and in some cases the phenomenon of drug resistance occurs, i.e. the tolerance of tumor cells to anti-tumor agents. Vinblastine, an alkaloid from *Catarantus roseus*, has been used as a drug against multiple myeloma (Costa et al., 1963) and Hodgkin's lymphoma (Meynard et al., 2020), and resistance to vinblastine has been observed in affected patients from multiple myeloma. Some new information on vinblastine-related drug resistance has been reported by studying P3X63Ag8.653 murine myeloma cells incubated with low concentrations of the drug (Laghezza Masci et al., 2023). The treatment selected cells resistant to vinblastine and demonstrated that the culture medium of these cells was capable of inducing resistance to the drug in cells not previously treated with the drug. Metabolomics analysis performed on untreated cells, resistant cells and induced drug-resistant cells after cultivation in resistant cell culture media clearly showed differences in the metabolome, thus opening perspectives for similar studies on human myeloma cells. Such investigations could suggest paying attention also to the compounds produced by the cells and secreted into the culture medium after low concentration treatment.

Active biomolecules as possible interfering agents of cellular metabolism and the autophagocytosis pathway.

Autophagy is a pathway used by cells to digest aged organelles and/or damaged cytoplasmic materials. During this process, intracellular materials are wrapped in double-layered membrane structures called "autophagosomes"; next step, autophagosomes fuse with lysosomes to form new functional structures called "autophagolysosomes", in which the digestion of organelles and/or cytoplasmic materials occurs (Kundu and Thompson, 2008). If the autophagy process does not occur regularly, cytoplasmic disorders and pathological conditions are observed inside the cells leading to many diseases such as metabolic syndromes, inflammatory disorders, cancer, cardiovascular diseases, neurodegeneration and aging (Arroyo et al., 2014). The autophagic process can be monitored by the LC3 protein, present in two isoforms called LC3B I and LC3B II respectively, which moves autophagosomes along the elements of the cytoskeletal apparatus; the two isoforms are considered descriptive of autophagic activity. LC3B I is present in the basal condition and is then converted to LC3B II during the formation of autophagosomes. When fusion of the autophagosome with the lysosome occurs, the LC3B II isoform will be degraded by lysosomal activity (Glick et al., 2010). The selection of biomolecules capable of interfering with cellular metabolism and the autophagocytosis pathway could lead to the development of specific drugs capable of combating some of the diseases mentioned. An approach to study the autophagocytosis pathway and the possible selection of interfering biomolecules has been reported (Bernardini et al., 2017) using the High-Content Screening technique (Klionsky et al., 2016). The effects of the treatments in the cellular systems were examined simultaneously using an automated confocal microscope and detecting different features of the cytoplasm; with this instrumentation it was possible to acquire images of lysosomes in treated living cells and evaluate the effects of biomolecules collected from different sources on the cellular acidic compartments and on the isoforms of the LC3 protein. The quantitative data extracted from the images were then used to determine specific parameters useful for evaluating the activity of the biomolecules.

Conclusions

Over time, humans have extensively used plants and other living sources as food and healing resources. At a scientific level there have been notable advances especially in the last three centuries and in recent times new investigative tools support human research activities. From the first observations of botanists and the analyses of chemists, a large group of researchers,

such as biologists, chemists, pharmacologists, doctors have improved their knowledge and, also supported by industries, are currently engaged in the research and evaluation of compounds of natural origin and their possible applications in different areas of human society. It is a contribution to the future of humanity and the sustainability of human progress, and bioactive compounds are expected to continue to play a useful role.

References

Achilli, Toni-Marie, and Meyer, Julia, and Morgan, Jeffrey R. 2012. "Advances in the formation, use and understanding of multi-cellular spheroids". *Expert opinion on biological therapy*, No. 12(10): 1347-1360. doi:10.1517/14712598.2012.707181.

Anke, H, and Sterner, O. 1991. "Comparison of the antimicrobial and cytotoxic activities of twenty unsaturated sesquiterpene dialdehydes from plants and mushrooms." *Planta medica*, No. 57(4): 344–346. https://doi.org/10.1055/s-2006-960114

Arroyo, Daniela S, and Gaviglio, Emilia A, and Peralta Ramos, Javier M, and Bussi, Claudio, *et al*. 2014. "Autophagy in inflammation, infection, neurodegeneration and cancer". *International Immunopharmacology,* No. 18: 55-65. doi:10.1016/j.intimp.2013.11.001.

Aru, Basak, and Gulderen, Yanikkaya Demirel. 2024. "Flow Cytometry: A Versatile and Powerful Tool for Drug Discovery and Development". *Pharmedicine Journal,* No. 1(1): 1-19. https://doi.org/10.62482/pmj.5.

Atanasov, Atanas G, and Zotchev, Sergey B, and Dirsch, Verena M, and International Natural Product Sciences Taskforce, and Claudiu, T Supuran. 2021. "Natural products in drug discovery: advances and opportunities". *Nature reviews. Drug discovery,* No. 20(3): 200-216. doi: 10.1038/s41573-020-00114-z.

Baez, Hernan, and Castro, Mario M, and Benavente, MA, and Kintz, Pascal, *et al*. 2000. "Drugs in prehistory: chemical analysis of ancient human hair." *Forensic science international,* No. 108(3): 173-9. doi:10.1016/s0379-0738(99)00204-2

Bailly, Christian. 2019. "Irinotecan: 25 years of cancer treatment". *Pharmacological Research,* No. 148: 104398. doi.org/10.1016/j.phrs.2019.104398.

Balabanova, Svetlana, and Rösing, Friedrich W, and Teschler-Nicola, Maria, and Strouhal, Eugen, *et al*. 1996. "Was nicotine used as stimulant already in the VI century AD from the Christian Sayala population?". *Journal of Paleopathology,* No. 8: 43-50.

Baker, Dwight, and Chu, Min, and Oza, Uma, and Rajgarhia, Vineet. 2008. "The value of natural products to future pharmaceutical discovery". *Natural product reports,* No. 24: 1225-1244. doi:10.1039/b602241n.

Behera, Bhaskar C, and Adawadkar, Bharati, and Makhija, Urmila V. 2003. "Inhibitory activity of xanthine oxidase and superoxide-scavenging activity in some taxa of the lichen family Graphidaceae". *Phytomedicine,* No. 10(6-7): 536-543. doi:10.1078/094471103322331511

Bernardini, Stefano, and Tiezzi, Antonio, and Laghezza Masci, Valentina, and Ovidi, Elisa. 2017. "Natural products for human health: an historical overview of the drug discovery approaches". *Natural Product Research,* 01-25. doi:dx.doi.org/10.1080/14786419.2017.1356839

Bernini, Roberta, and Carastro, Isabella, and Palmini, Gaia, and Annalisa, Tanini, *et al*. 2017. "Lipophilization of Hydroxytyrosol-Enriched Fractions from *Olea europaea* L. Byproducts and Evaluation of the in Vitro Effects on a Model of Colorectal Cancer Cells". *Journal of Agricultural and Food Chemistry*, No. 65. doi:10.1021/acs.jafc.6b05457.

Bobek, P, and Ginter, E, and Jurcovicová, M, and Kuniak, L. 1991. "Cholesterol-Lowering Effect of the Mushroom *Pleurotus ostreatus* in Hereditary Hypercholesterolemic Rats." *Annals of nutrition & metabolism*, No. 35(4): 191-195. https://doi.org/10.1159/000177644.

Borchardt, John. 2002. "The Beginnings of Drug Therapy: Ancient Mesopotamian Medicine". *Drug news & perspectives*. No. 15. doi:187-192. 10.1358/dnp.2002.15.3.840015.

Brzin, Joze, and Rogelj, Boris, and Popovic, Tatjana, and Štrukelj, Borut, and Ritonja, Anka. 2000. "Clitocypin, a New Type of Cysteine Proteinase Inhibitor from Fruit Bodies of Mushroom *Clitocybe nebularis*." *The Journal of biological chemistry*, No. 275: 20104-20109. doi:10.1074/jbc.M001392200.

Brockmann, Maxwell C., and American Institute of Chemical Engineers. 1970. *The History of Penicillin Production*. Edited by Albert Lawrence Elder. New York: American Institute of Chemical Engineers.

Buss, AD, and Cox, B, and Waigh, RD. 1995. "Natural Products as Leads for New Pharmaceuticals. Antiparasitic drugs". In Burger's medicinal chemistry and drug discovery, edited by Abraham, Donald J, 886-891. New York (US): Wiley-Interscience.

Cao, Qi-zhen, and Lin, Zhi-Bin. 2006. "*Ganoderma lucidum* polysaccharides peptide inhibits the growth of vascular endothelial cell and the induction of VEGF in human lung cancer cell." *Life sciences*, No. 78: 1457-1463. doi:10.1016/j.lfs.2005.07.017.

Caroleo, Maria Cristina, and Plastina, Pierluigi, and Fazio, Alessia, and La Torre, Chiara, *et al*. 2021. "Olive Oil Lipophenols Induce Insulin