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Bioactive Molecules Profile from Natural Compounds

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.68643>

Abstract

Currently, wide world research is focused on sustainable development and the demand for innovative clean technologies, nevertheless natural potential reconsideration could represent a viable solution for the identification and design of new pharmacological agents from renewable resources. The main reason consists of special properties of these natural derivatives: immunomodulating activity with continuously perfectible selectivity and efficiency. Plants and herb extracts have been used for centuries as traditional medicines, throughout the entire world. Romanian phytotherapy represents practically a very important part of our traditional knowledge and heritage. Therapeutic properties of plant active principles still continue to be the subject of many researches. In this chapter, an overview of plant bioactive molecules from the perspective of modern phytochemistry is presented. A special part is devoted to a very special medicinal plant, *Viscum album*, in particular identification of amino acids and thionins from mistletoe.

Keywords: phytochemicals, secondary metabolites, analytic methods

1. Introduction

Since ancient times, people have searched and found in nature remedies for various diseases [1, 2]. Romanian tradition pays a special attention to plants which attributes them the properties of living beings (soul, feeling, hearing and sight). Also there is an extraordinary relation between human beings and nature, an almost mystical interdependence. Most often the healing herbs were considered sacred. Phytotherapy origins are lost in the mists of time. In Romania, the traditional medicine has a very long history. Platon, Herodot and Pedanos Dioscoride have mentioned about the herbal medical system from Dacia and medicinal plants

used by our ancestors [1]. In Romanian tradition, there is a ritual harvesting these herbs which requires strict compliance with the optimal schedule at a specified date and time. Such is the case of belladonna (*Atropa belladonna*) that is harvested on full moon only from April–May period, before Pentecost. *Medicago falcate* known as earth vortex must be collected only on harvest time. *Melilotus officinalis* is plucked only on Sanziene holiday and on Cross day, two important Romanian holidays. It is believed that after this period the plant loses its properties. Romanian traditional medicine involves a very large number of heal plants: twigs, buds, bark and leaves of trees (alder, sambucus), flowers, seeds, stems or roots from plants. Some of the healing herbs were specific to Romanian herbal medicine: *Salicornia herbacea*, *Anchusa officinalis*, *Actaea spicata*, *Symphytum officinale*, *Verbascum thapsus*, *Urtica dioica*, *Cicuta virosa*, *Typha angustifolia*, *Chelidonium majus*, *Bryonia alba* L., *Thymus vulgaris* L., *Alisma plantago-aquatica* L., *Hyoscyamus niger* L., *Verbascum phlomoides* L., *Achillea millefolium* L., *Veratrum album*, *Clematis vitalba* L., *Potentilla reptans* L., *Lappa maior* Garthn., *Datura stramonium* L., *Dipsacus pilosus* L., *Erythraea centaurium* Pers., *Mentha piperita* L., *Cynoglossum officinale* L., *Lithospermum arvense* L. and *Galim verum* [3]. But then their use was spread throughout Balkan areal and Europe. Currently, it is widely used for *Symphytum officinale* for its anti-inflammatory and wound healing activity. Withal, this plant has a high content of allantoin, one of the active principles of the plant it became more important as an ingredient in cosmetics [4–7].

Recent studies on medicinal plants assigned the therapeutic capacity of medicinal plants to their complex structure composed mainly from highly bioactive compounds, minerals, vitamins, etc. [2].

Generally, medicines contain just one active substance, synthetically, whereas medicinal plants are practically a mixture of over dozens or even hundreds of chemicals that act synergistically [2–3]. Moreover, medicinal plants contain a large amount of vitamins and minerals, easily assimilated by human body. Many recent studies demonstrate that vitamins and minerals obtained through chemical synthesis have not the same beneficial effect as similar natural products. It may be due to the fact that in natural products there is a synergistic and complementary action between vitamins, minerals and enzymes, while synthetic compounds (vitamins or minerals) are isolated and even obtained as a different enantiomeric form [8–10]. On the other hand, drugs present other major disadvantages compared with medicinal plants: (i) various side effects; (ii) contraindications; (iii) interactions with other substances; (iv) drug resistance (drug dependence); (v) expensive and (vi) long time consuming research [8]. In comparison, natural compounds present a superior structural diversity, complex structure and multiple stereocenters [10–12]. These are just few arguments that may tilt the scales in favor of herbal medicines. Moreover, World Health Organization (WHO) aims to increase the integration of traditional medicine in order to improve health care system [13].

2. Plant metabolite

Paramount importance of botanic products for humanity is due mainly to their phytochemicals, active principles with therapeutic properties. Several studies have investigated these plant-derived compounds [14–19]. Depending on the role they hold in living organisms,

natural substances are divided in the next major categories: (i) *primary metabolites*, molecules common to all biological systems (proteins, fats, sugars) and (ii) *secondary metabolites*, compounds that could be specific for different species as a direct result of the evolution process of a particular phylogenetic group [16, 18–20]. **Figure 1** shows a schematic representation of plant metabolites [16–20].

Bioactive molecules are basically those secondary metabolites exhibiting therapeutic, preventing, toxicological and immunostimulating activity [16–20]. The most known plant-derived bioactive compounds are presented in **Figure 2**.

Figure 1. Plant metabolites.

Figure 2. Schematic representation of plant bioactive compounds.

Biological activity of these compounds has been extensively investigated in particular in the last decades [4–32]. Thus, it demonstrated that there is a close connection between the chemical structure of the natural active principles (functional group types, number and position related to carbon skeleton, substitution in aromatic ring, stereochemistry, side chain length, saturation, etc.) [17, 20, 22, 25, 27, 34]. The role of metabolites in human organism is briefly presented in **Table 1**. And some examples of these compounds are shown in **Table 2**.

Compound type	Pharmacological properties
Terpenoid	Antimicrobial, antiviral, anthelmintic, antibacterial, anticancer, antimalarial, anti-inflammatory [15, 34]
Phenolics acids	Anticarcinogenic and antimutagenic, anti-inflammation and anti-allergic [16, 20, 25, 31–35]
Alkaloids	Antispasmodic, antimalarial, analgesic, diuretic activities, local anesthetic, antihypertensive, antiasthma, antimalarials, diuretic, bactericidal [14–16, 20, 21]
Flavonoids	Antioxidant activity, cardiovascular protective, anti-inflammatory, hepatoprotective, antiviral, antibacterial [20, 22–24, 34]
Saponins	Antitumor, antiviral, antifungal, anti-inflammatory, immunostimulant, antihypoglycemic, antihepatotoxic and hepatoprotective, anticoagulant, neuroprotective, antioxidant [16, 20, 24–27, 34]
Tannins	Antioxidant, anti-carcinogenic, diuretics, hemostatic, anti-mutagenic, metal ion-chelators, antiseptic, [14, 16, 20, 25, 28–32]

Table 1. Biologic activity of main groups of natural compounds.

Secondary metabolites	Important molecules	References
Alkaloids	Caffeine, piperine, atropine, berberine, morphine, quinine, cocaine, nicotine, strychnine, codeine, ephedrine, dopamine, serotonin, vinblastine, vincristine, brucine, capsaicin, solanine, tomatine, choline, etc.	[15, 21, 34]
Terpenes	<i>Hemiterpene</i> : isoprene, isovaleric acid <i>Monoterpene</i> : limonele, eucalyptol, menthol, nerol, citral <i>Sesquiterpene</i> : zinziberene, farnesol <i>Diterpene</i> : cafestol, retinal, retinol <i>Sesterterpenes</i> : bulgarene, farnesol, lindarene <i>Triterpene</i> : provitamin A, betulin, cymarín <i>Tetraterpene</i> : lycopene, α si β carotenoids <i>Polyterpene</i> : vitamin E, gutta-percha	[15, 34]
Flavonoids	<i>Flavones</i> : luteolin, diosmetin, apigenin <i>Flavonols</i> : quercetin, myricetin, rutin, kaempferol <i>Flavanones</i> : hesperetin, naringenin <i>Flavanonol</i> : silymarin, taxifolin <i>Isoflavones</i> : daidzin, genistin <i>Anthocyanidin</i> : cyanidin, delphinidin, peonidin, petunidin	[15, 22, 23]

Secondary metabolites	Important molecules	References
Phenolic acids	Cinnamic acid, benzoic acid, ferulic acid, coumaric acid, caffeic acid, salicylic acid, gallic acid	[15, 33]
Saponins	Panaxadiol, diosgenin	[15]

Table 2. Some well-known examples of plant metabolites.

3. Profiling of plant bioactive molecule

Achievement of the natural plant bioactive molecules profile involves more consecutive stages (**Figure 3**) [14, 17, 18].

3.1. Selection of plant species

First and foremost stage is required to evaluate the existing ethnomedicinal studies, chemotaxonomical data regarding a particular medicinal plant, information collected from different historic documents, traditional knowledge from even local quacks and specialists [14, 37].

Figure 3. Flowchart of plant bioactive molecules profiling.

3.2. Collection and identification of plant species

This represents a key stage required to afford a reliable profile of natural active principles. And involve the next steps:

- (a) Procurement of botanic component only from sources with guaranteed good agriculture and collection practices. An essential step demand to investigate a possible microbial, pesticide or heavy metals contaminations to avoid adversely affect the results of the chemical screening of bioactive metabolites, increased the time and cost of studies [18, 36, 37]. **Table 3** presents the main analytical techniques used to detect a possible plant contamination.
- (b) Plant taxonomic or genetic identification [18, 36, 37]. A modern method for authentication the botanic precursor use genomic analysis (DNA barcoding method) [38]. Research has been shown that biodiversity and plant growth environmental conditions (temperature, humidity, soil physic and chemical properties) could influence the bioactive molecules profile [39].

3.3. Preparation of plant material (drying, micronisation, etc.)

The botanical material processing is needed to avoid the degradation of plant bioactive compounds [14]. The drying is recommended to be performed in areas-controlled atmosphere (absence of humidity, well-ventilated, constant temperature).

The dried botanic material is subjected to micronization process through mechanical techniques. The other methods of plant sample preparation involve: (i) botanic material homogenization or (ii) plant maceration [14, 39, 42].

This step aims to minimize the sample particle dimensions and thus to enhance the extraction yield [14].

3.4. Extraction and isolation of bioactive molecules

This is the key stage in evaluation of natural bioactive compounds.

- (a) *Extraction and separation techniques:* In literature, there are many studies on extraction of certain groups of plant metabolites. However, the selectivity of conventional extraction methods (soxhlet extraction, hydrodistillation, maceration, percolation, steam distillation, etc.)

Plant contamination assay	Analytical method
Heavy metals	Atomic absorption spectroscopy, ICP-MS, etc.
Pesticide or/and herbicide residues	GC-MS, mass spectrometry, HPLC-MS, etc.
Microbial content	HPLC-MS, etc

Table 3. Plant contamination: chemical assays.

are at least moderate and economically inefficient (energy, hazardous reagents consumption, time and temperature) [18, 39–42]. The other main disadvantages of these techniques are (i) not environment friendly; (ii) high possibility of degradation of thermostable active principles and (iii) additional steps (extract concentration, cleanse) [39–42]. Advanced extraction processes (solid-phase extraction, ultra-sound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, pulsed electric field extraction, pressurized liquid extraction, enzyme-assisted extraction, surfactant-mediated extraction) have minimized many of these shortcomings. Usually, the separation of a particular group of bioactive compounds from a complex natural product required a selective separation strategy based on phytochemicals partition in several different polarity solvents [43]. Nevertheless, natural product chemistry research concerns the development of new and highly efficient extraction techniques. Recent studies have reported that calixarenes could represent an attractive opportunity in this regard [44].

- (b) *Isolation methods*: The physical properties (solubility, molecular weight, stability, dipole moment, etc.) of targeted bioactive compounds are essential for an efficient isolation method [39, 41, 42]. Another important factor is the nature of extraction solvent [39]. Generally, based on existing databases, the plant metabolites isolation are carried out through chromatographic methods: thin chromatography (TLC), flash chromatography, high performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), gas chromatography (GC) or Fourier transform infrared spectroscopy (FT-IR) [14, 39, 41, 42]. A biological material previously uninvestigated and is in demand to develop an appropriate isolation procedure that require following additional steps: (i) phytochemical evaluation; (ii) bioassay (immunoassay (monoclonal antibodies) [14, 39].

3.5. Identification and structural elucidation (chemical screening)

This is the forefront but also the most difficult step in natural product chemistry. Achievement of the bioactive molecules complete profile requires the cutting-edge technology and advanced knowledge specialists. Investigation on new natural compounds entails a larger work volume determined mainly by the absence of plant scientific data [14, 39, 45–48]. Plant bioactive molecules profiling is based on various spectroscopic techniques, advanced chromatographic (hyphenated techniques) methods and a complete morphostructural characterization procedure using X-ray crystallographic techniques, polarimetry and electronic microscopy (**Table 4**) [14, 39, 45–48]. An optimal strategy based on high-tech technology provides fast and highly efficient complete structural information about the targeted compounds [39, 42, 47, 48]. **Table 5** shows the main analytical techniques applied in natural bioactive compounds chemical screening [14, 39, 45–48].

3.6. Biological and pharmacological screening

There are various methods designed to investigate the biological activity of a targeted natural compounds. An optimal procedure must fulfill several criteria: fast, simple, reliable, high sensibility and selectivity, availability and low cost. Bioactivity evaluation for a

Spectroscopic methods	UV-Vis spectroscopy
	Fourier transform infrared spectroscopy
	Mass spectroscopy:
	(a) Electron impact mass spectrometry (EIMS) (b) Chemical ionization mass spectrometry (CIMS) (c) Electrospray ionization mass spectrometry (ESIMS) (d) Electrospray ionization mass spectrometry (ESIMS) (e) Fast atom bombardment mass spectrometry (FABMS)
Chromatography methods	Nuclear Magnetic Resonance (NMR) spectroscopy:
	(a) One-dimensional techniques: ¹ H-NMR, ¹³ C-NMR, ¹³ CDEPT, ¹³ CPENDANT, ¹³ C J mod.
	(b) Two-dimensional techniques: ¹ H- ¹ H COSY, ¹ H- ¹ H DQF-COSY, 1H-1H COSY-Ir, 1H-1H NOESY, ¹ H- ¹ H ROESY, ¹ H- ¹ H TOCSY, ¹ H- ¹³ C HMBC, ¹ H- ¹³ C HMQC, ¹ H- ¹³ C HSQC,HSQCTOCSY
	Gas-chromatography: GC, GC-MS, GC-TOF-MS; GC-MS/MS, two-dimensional GC coupled with mass spectrometry (GC×GC-MS), GC-FTIR, GC-NMR
Other analytic techniques	Liquid chromatography: LC/UV; LC/MS; LC/UV/MS; LC/MS-MS; LC/NMR, LC-UV-DAD, HPLC-NMR
	XRD; TEM; polarimetry

Table 4. A brief overview of bioactive molecules profiling tools [39, 42, 47, 48].

Plant sample	Propose structure	Abbreviation	SIM (selected-ion monitoring)
V ₁ (hexane)	Cystine	C-C	41, 42
	Glutamic acid	Glu	38, 40
	Phenylalanine	Phe	56, 57
	Ornithine	Orn	59,60,61
	Histidine	His	84, 89
	Tyrosine	Tyr	61, 63, 94
	Glycine	Gly	116, 74
	Homoserine	HSER	102,128, 143
	Asparagine	Asn	155, 69
	Isoleucine	Ile	171, 129
	Valine	Val	158, 116
	Threonine	Thr	160, 101
	β-Alanine	β Ala	158, 98
	Valine	Val	158,72
	β-Alanine	β Ala	129, 158, 98
	Homoserine	HSER	102, 128, 143
	Asparagine	Asn	155, 69

Plant sample	Propose structure	Abbreviation	SIM (selected-ion monitoring)
V ₂ (CCl ₄)	Asparagine	Asn	155, 69
	Cystine	C-C	41,42
	Alanine	Ala	130, 70
	Glutamic acid	Glu	38, 40
	Ornithine	Orn	59,60,61
	Tryptophan	Trp	130
	β-Alanine	β Ala	129, 158, 98
	Phenylalanine	Phe	56, 57
	Tyrosine	Tyr	61, 63, 94
	Homoserine	HSER	102,128, 143
	Valine	Val	158,72
	Lysine	Lys	170, 129
	Glycine	Gly	116, 74
	Isoleucine	Ile	170, 130
	Hystidine	Hys	84, 87
V ₃ (petroleum ether)	Glutamic acid	Glu	38, 40
	Cystine	C-C	41,42
	Phenylalanine	Phe	56, 57
	Glycine	Gly	116, 74
	Leucine	Leu	172, 86
	β-Alanine	β Ala	129, 158, 98
	Isoleucine	Ile	170, 130
	Cysteine	Cys	248, 162, 206
	Tyrosine	Tyr	61, 63, 94
	Hystidine	Hys	84, 87
	Glutamine	Gln	84, 187
	Lysine	Lys	170, 129
	Tryptophan	Trp	130
	Valine	Val	158,72
	Aspartic acid	Asp	216, 130
	Methionine sulfoxide		229,182,138
	S-Carboxymethyl-cysteine		144,203,262
	Proline-hydroxyproline (dipeptide)	PHP	156, 186
	Lysine-alanine (dipeptide)	LYS-ALA	170, 224, 153
	3-Methyl-cysteine	1MHIS	172,259,130
	Arginino succinic acid	ARG-SUC	441, 326
	Methionine	Met	203, 277
	Cystathionine	CTH	203, 272

Plant sample	Propose structure	Abbreviation	SIM (selected-ion monitoring)
V ₄ (acetone)	Cystine	C-C	41,42
	Glutamic acid	Glu	38, 40
	Phenylalanine	Phe	56, 57
	β-Alanine	β Ala	129, 158, 98
	Ornithine	Orn	59,60,61
	Glycine	Gly	116, 74
	Isoleucine	Ile	170, 130
	Histidine	Hys	84, 87
	Glutamine	Gln	84, 187
	Valine	Val	158,72
	Tyrosine	Tyr	61, 63, 94
	Lysine	Lys	170, 129
	Homoserine	HSER	102,128, 143
	Proline-hydroxyproline (dipeptide)	PHP	156, 186
	3-Methyl-cysteine	1MHIS	172,259,130
	Homocysteine	HCYS	142, 203
	Glycyl-glycine (dipeptide)	Gly-Gly	117, 144, 201

Table 5. Compounds identified through GC-MS analysis.

plant extraction (plant fraction) is usually performed through *in vitro* or/and *in vivo* studies [14, 49, 50]. Most often, *in vitro* studies are focused on the evaluation of specific cell biology (cell count, growth rate, metabolic rate, cell function and protein expression). *In vitro* tests are conducted on various animal or human cell cultures, enzymes, depending on targeted natural compound biological activity [14, 49, 50]. For instance, the bioassays for antitumor activity are conducted on tumor experimental models. Complementary, the immunological activity on normal cell culture should be monitored. The cells will be analyzed by fluorescence microscopy and will be quantified to establish the degree of apoptosis and implicitly the cell viability. Also, the time-lapse video microscopy can be used to evaluate the bioactive phytochemicals [43]. The *in vivo* biotests are applied on animals (mice, rats, pigs, etc.).

Natural compounds bioassay can be demonstrated also using computational chemical methods: quantitative structure-activity relationship (2D or 3D QSAR) and structure-activity relationship (SAR) [75, 76].

Regarding the antioxidant activity of natural compounds, literature demonstrates the existence of a considerable number of studies using two analytical techniques: electron spin resonance (ESR) and chemiluminescence. But the obtained results depend on the type of reactant (specific free radical) used [51]. Electrochemistry, especially by the instrumentality of voltammetry has

been shown to be a useful method for the investigation of the antioxidant activity of different targeted compounds [52].

4. Natural compounds in *Viscum album* as an example of medicinal plant

One of the most renowned medicinal plants is *Viscum album* L., which has very different applications: tonic, cardiogenic, antiviral, cancer, etc. In different European countries, mistletoe extracts are prepared and commercially available (*Iscador*, *Isorel*, *Eurixor*, *Plenesol*, *Vysorel*, *Lektinol*, *Helixor*, etc.) as alternative treatment for cancer therapy [53–58].

First information on the use of this plant for its benefits on the human body dates back to ancient times. The druids and Celts considered as sacred mistletoe that grows on oak. Over time, peoples were attributed a special symbolism to this evergreen plant: immortality, knowledge, wisdom, universal panacea, love, fortune, fertility, etc. [54, 57]. There are considered that magical properties of mistletoe are kept only if the complied both the collection ceremony: a golden knife in a special moment of day before full moon, on right period (summer or winter solstice) [54].

In traditional medicine, *Viscum* are used for various health benefits: poison antidote, anti-age, anti-inflammatory, fertility, antitumor, headaches, preventing epilepsy, cure for plague, erysipelas, etc. [53–55].

Many studies have been carried out for determination of the outstanding biological effects: antiproliferative activity, antitumor activity, antiviral activity, cardiovascular, immunostimulant and antidiabetic [56, 58–65]. But the extremely complex chemical composition of this plant has not been precisely determined yet. Nevertheless, several secondary metabolites such as flavonoids, alkaloids, steroids, terpenoids were detected [66]. However, research has demonstrated that *viscum* chemical composition varies depending on (i) the type of host tree on which it grows (oak, maples, acacia, robinia, poplar, etc.), (ii) time of harvesting, (iii) environmental conditions and (iv) extraction method [56, 67].

The attempts to establish the compounds responsible for biological, immunomodulating and cytotoxic activity had targeted especially the lectins and viscotoxins as active components [56, 67]. Nevertheless, these compounds represent only a small content of percent from the entire plant peptide content which is not fully understood in terms of chemical structure and biological activity. Relatively recent research had emphasized on the presence of other peptide derivate, *viscumamide* with antitumor activity [68]. However, there are still many compounds pharmacologically active that can be found. Continuous development of analysis techniques can provide important information about new highly bioactive compounds isolated from plant extracts.

4.1. Importance of natural small peptide

From the multitude of classes of biomolecules isolated from natural compounds, a special attention has been given to amino acids and small peptides due to their remarkable properties

(high solubility, strong antioxidant, reduce high blood pressure, analgesic, anti-tumor, immunomodulatory, etc.). In addition, these biologically active compounds have various applications in pharmacology, cosmetics, sports and food.

In plants, these biomolecules are involved also in defense mechanisms against various classes of pathogens (bacteria, fungi, parasites, etc.) [69, 70].

Given that cancer is the second leading cause of death in European countries, and one of the most imminent health problems in the developed world [71–73], there is an overwhelming interest for new efficient antitumor agents with high bioavailability and minimal side effects. In this context, research on plant bioactive molecules with putative antitumor activity is even more justified.

Thionins represent a special class of small peptide with multiple disulfide bonds [43, 68, 69]. They have shown cytotoxicity and antitumor activity [69, 70]. Research has reported that mistletoe contains several types of thionins: viscothionin A1, viscothionin A2, viscothionin A3, viscothionin B, viscothionin C1, viscothionin D, viscothionin E, viscothionin P1 [69, 70].

4.2. Determination of amino acids and thionins from *Viscum album*

In an effort to detect the amino acids and thionins from *Viscum album* a selective partition strategy based on solvents with different polarities (methanol, hexane and carbon tetrachloride) was developed [43]. The plant material (*Viscum album* leaves and young leaves from *Quercus robur*) was obtained from a collection taken in December 2015 in Timis, Romania. Plant sample was identified at Victor Babes University of Medicine and Pharmacy Timisoara. The botanical material was dried and then finely ground in a ball mill. A plant sample (3 g) was placed in a 100 mL volumetric flask containing 50 mL of methanol. The result mixture was sonicated for 60 min at 40°C, with a frequency of 50 kHz. Then the solution was filtered through a 0.30 µm pore size filter and subsequently extracted with the following organic solvents: *n*-hexane (V_1) and carbon tetrachloride and (V_2). The separation of thionins was carried on the next experiment: 2 g of sample was extracted successively with petroleum ether (30 mL) and acetone (30 mL) [43]. Identity of the compounds from the obtained viscum fractions: V_1 (hexane), V_2 (CCl_4), V_3 (petroleum ether) and respectively, fraction V_4 (acetone) was performed using GC-MS and TOF MS methods.

4.3. GC-MS analysis

The GC-MS chromatograms for mistletoe extract fraction V_1 – V_5 are presented in **Figure 4(a)–(d)**.

The results of design isolation strategy based on different solvent polarity were analyzed through GC-MS [43]. The identified compounds are presented in **Table 5**; after a careful comparison with spectral database, NIST/NBS was used to compare the results of analysis [43].

4.4. TOF-MS analysis

The mass spectra of mistletoe fractions V_1 – V_4 (acquired in positive ion mode, in a mass range of 100–3000 m/z) are presented in **Figure 5(a)–(d)**.

Figure 4. TIC of (a) V_1 extract, (b) V_2 extract, (c) V_3 extract and (d) V_4 extract.

Figure 5. Positive ion mode TOF-MS of (a) V_1 extract, (b) V_2 extract, (c) V_3 extract and (d) V_4 extract.

4.5. FT-IR spectroscopy

The solid (fine grounded) sample of mistletoe was analyzed also through FT-IR spectroscopy (**Figure 6**). It has been aimed to identify the absorptions bands specific to amino acids and peptides from: (i) 3400 cm^{-1} (O-H and N-H bonds); (ii) $3330\text{--}3130\text{ cm}^{-1}$ (NH_3^+ groups); (iii) symmetric absorption at $2080\text{--}2140\text{ cm}^{-1}$ or $2530\text{--}2760\text{ cm}^{-1}$; (iv) $1500\text{--}1600\text{ cm}^{-1}$ (ammonium group deformation vibrations); (v) $1610\text{--}1660\text{ cm}^{-1}$ (carboxylate group); (vi) $1724\text{--}1754\text{ cm}^{-1}$ (carbonyl vibrations) and (vii) vibrations bands characteristic for thionins ($1687, 1675, 1663, 1654, 1644, 1632, 1621, 1611$) [45, 74].

The FT-IR spectra were recorded using a Universal ATR accessory (UATR) and mistletoe samples 20 mg and 30 mg, respectively, mixed with KBr.

From the spectra analysis, the presence of bands specific to amino acids, thionins and peptides can be noticed.

Figure 6. The FT-IR spectra for the mistletoe sample.

5. Conclusions

The collective results suggest that chosen separation solvent and analytic strategies are efficient for isolation and identification of targeted natural compounds from mistletoe sample. Further studies on mistletoe extract are necessary to gain insight into the complete bioactive molecules profile with high antitumor activity.

Continuous development of analysis techniques can provide important information about highly bioactive molecules isolated from natural compounds. Particular importance must be paid to the choice of optimal separation methods which must be simple but highly selective and efficient for separation of a certain class of natural metabolites. A special emphasis has been given to identify the peptides because it was considered that nature of amino acids, their quantity in plant and the ratio to known peptides for their high bioactivity may be relevant to their anticancer action. Research on small peptide with pharmacological activity continues to be a topic of great interest to the current science due to their special high biological activity, chemical stability, bioavailability, etc. From this perspective, further research will allow to predict the formulation of the peptide profile from natural extract with a specific biological effect with application in cancer prevention or therapy.

Acknowledgements

We like to thank Dr. Andrei Bunaciu for his contribution to this chapter.

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References

- [1] Danciu ET, Dusca AI. The Spirituality of the Geo-Dacian People—between Truth and Legend. *Revista de Stiinte Juridice*. 2008;141
- [2] Sandberg F, Corrigan D. *Natural Remedies. Their Origins and Uses*. New York: Taylor & Francis; 2001
- [3] Crisan IH. *Medicina in Dacia*. Ed. Dacica; 2013. ISBN 978-973-88076-2-4
- [4] Bojor O. *Ghidul plantelor medicinale si aromatice de la A la Z*, Ed. Fiat Lux; 2003. ISBN 973-9250-68-8

- [5] Neagu E, Roman GP, Radu GL. Antioxidant capacity of some *Symphytum officinalis* extracts processed by ultrafiltration. *Romanian Biotechnological Letters*. 2010;**15**(4):5505–5511
- [6] Alkan FU, Anlas C, Ustuner O, Bakirel T, Sari AB. Antioxidant and proliferative effects of aqueous and ethanolic extracts of *Symphytum officinale* on 3T3 Swiss albino mouse fibroblast cell line. *Asian Journal of Plant Science and Research*. 2014;**4**(4):62–68
- [7] Thornfeldt C. Cosmeceuticals containing herbs: Fact, fiction, and future. *Dermatologic Surgery*. 2005;**31**:873–880
- [8] Taylor L. *The Healing Power of Rainforest Herbs*. Square One Publishers Inc, New York, USA. 2005. ISBN: 0-7570-0144-0
- [9] Clement B. *Nutri-Con: The Truth About Vitamins & Supplements. The Vitamin Myth Exposed*. Hippocrates Health Institute & OCA; 2005. <https://www.organicconsumers.org/news/nutri-con-truth-about-vitamins-supplements>
- [10] Nguyen LA, He H, Pham-Huy C. Chiral drugs: An overview. *International Journal of Biomedical science*. 2006;**2**:85–100
- [11] Lahlou M. The success of natural products in drug discovery. *Pharmacology & Pharmacy*. 2014;**4**:17–31
- [12] Phillipson JD. Phytochemistry and medicinal plants. *Phytochemistry*. 2001;**56**:237–243
- [13] World Health Organization (WHO). *Traditional Medicine Strategy 2014–2023*. Hong-Kong, China: World Health Organization; 2013. pp.1-78 1-78. ISBN 9789241506090
- [14] Chikezie PC, Ibegbulem CO, Mbagwu FN. Bioactive principles from medicinal plants. *Research Journal of Phytochemistry*. 2015;**9**(3):88–115
- [15] Shakya AK. Medicinal plants: Future source of new drugs. *International Journal of Herbal Medicine*. 2016;**4**(4):59-64
- [16] Capasso F, Gaginella TS, Grandolini G. *Phytotherapy—A Quick Reference to Herbal Medicine*. Berlin Heidelberg: Springer-Verlag; 2003. ISBN 978-3-540-00052-5
- [17] Soetan KO, Aiyelaagbe OO. The need for bioactivity-safety evaluation and conservation of medicinal plants—A review. *Journal of Medicinal Plants Research*. 2009;**3**(5):324-328
- [18] Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM. Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*. 2013;**117**:426-436
- [19] Dias DA, Urban S, Roessne U. A historical overview of natural products in drug discovery. *Metabolites*. 2012;**2**(2):303–336
- [20] Anulika NP, Ignatius EO, Raymond ES, Osasere OI, Abiola AH. The chemistry of natural product: Plant secondary metabolites. *International Journal of Technology Enhancements and Emerging Engineering Research*. 2016;**4**(8):1–8. ISSN 2347-4289
- [21] Woolley JG. Plant alkaloids. In: *Encyclopedia of Life Sciences*. Nature Publishing Group, John Wiley & Sons. 2001. pp. 1–11

- [22] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*. 2013:Article ID 162750
- [23] Falcone Ferreyra ML, Rius SP, Casati P. Flavonoids: Biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science-Plant Physiology*. 2012;**3**:Article 222
- [24] Negi JS, Negi PS, Pant GJ, Rawat SM, Negi SK. Naturally occurring saponins: Chemistry and biology. *Journal of Poisonous and Medicinal Plant Research*. 2013;**1**(1):001–006
- [25] Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry*. 2013;**1**(6):168–182
- [26] Kareru PG, Keriko JM, Gachanja AN, Kenji GM. Direct detection of triterpenoid saponins in medicinal plants. *African Journal of Traditional, Complementary and Alternative Medicines*. 2008;**5**(1):56–60
- [27] Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anti-cancer agents. *Fitoterapia*. 2010;**81**:703–714
- [28] Hassanpour S, Maheri-Sis N, Eshratkhah B, Baghbani Mehmandar F. Plants and secondary metabolites (Tannins): A review. *International Journal of Forest, Soil and Erosion*. 2011;**1**(1):47–53
- [29] Khanbabaee K, van Ree T. Tannins: Classification and definition. *Natural Product Reports*. 2001;**18**:641–649
- [30] Breitmaier E. Terpenes. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA; 2006. ISBN: 3-527-31786-4
- [31] Lattanzio V. Phenolic compounds: Introduction. In: Ramawat KG, Merillon JM, editors. *Natural Products*. Berlin Heidelberg: Springer-Verlag; 2013. pp.1543–1580
- [32] Balasundram N, Sundram K, Samman S. Analytical, nutritional and clinical methods—Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*. 2006;**99**:191–203
- [33] Robbins RJ. Phenolic acids in foods: An overview of analytical methodology. *Journal of Agricultural and Food Chemistry*. 2003;**51**:2866–2887
- [34] Kabera JN, Semana E, Mussa AR, He X. Plant secondary metabolites: Biosynthesis, classification, function and pharmacological properties. *Journal of Pharmacy and Pharmacology*. 2014;**2**:377–392
- [35] Ozcan T, Akpinar-Bayazit A, Yilmaz-Ersan L, Delikanli B. Phenolics in human health. *International Journal of Chemical Engineering and Applications*. 2014;**5**(5):393–396
- [36] van Breemen Richard B, Fong Harry HS, Farnsworth NR. The role of quality assurance and standardization in the safety of botanical dietary supplements. *Chemical Research in Toxicology*. 2007;**20**(4):577–582
- [37] Schwikkard SL, Mulholland DA. Useful methods for targeted plant selection in the discovery of potential new drug candidates. *Planta Medica*. 2014;**80**(14):1154–1160

- [38] Balachandran KRS, Mohanasundaram S, Sathishkumar R. DNA barcoding: A genomic-based tool for authentication of phytomedicinals and its products. *Botanics: Targets and Therapy*. 2015;**5**:77–84
- [39] Sarker SD, Latif Z, Gray AI. *Natural products isolation*. 2nd ed. New Jersey, USA: Humana Press Inc; 2006. ISBN 1-58829-447-1
- [40] Segneanu AE, Macarie CA, Pop RO, Balcu I. Combined microwave–acid pretreatment of the biomass. In: Shaukat SS, editor. *Progress in Biomass and Bioenergy Production*. Croatia: In Tech; 2011. pp.223–238. ISBN 978-953-307-491-7
- [41] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*. 2011;**8**(1):1–10
- [42] Kaufmann B, Christen P. Recent extraction techniques for natural products: Microwave-assisted extraction and pressurized solvent extraction. *Phytochemical Analysis*. 2002;**13**(2):105–113
- [43] Segneanu AE, Grozescu I, Cziple F, Berki D, Damian D, Niculite CM, Florea A, Leabu M. *Helleborus purpurascens*—Amino acid and peptide analysis linked to the chemical and antiproliferative properties of the extracted compounds. *Molecules*. 2015;**20**:22170–22187
- [44] Segneanu AE, Damian D, Hulka I, Grozescu I, Salifoglou A. A simple and rapid method for calixarene-based selective extraction of bioactive molecules from natural products. *Amino Acids*, Springer. 2016;**48**:849–858
- [45] Neda I, Vlazan P, Pop RO, Sfarloaga P, Grozescu I, Segneanu A-E. Peptide and amino acids separation and identification from natural products. In: Krull IS, editors. *Analytical Chemistry*. Croatia: Intech; 2012. pp.135–146. ISBN 978-953-51-0837-5
- [46] Segneanu AE, Gozescu I, Dabici A, Sfirloaga P, Szabadai Z. Organic compounds FT-IR spectroscopy. In: Uddin J, editor. *Macro to Nano Spectroscopy*. Croatia: InTech; 2012. pp.145–164. ISBN 978-953-51-0664-7
- [47] Guo X, Lankmayr E. Hyphenated techniques in gas chromatography. In: Mohd MA, editor. *Advanced Gas Chromatography—Progress in Agricultural, Biomedical and Industrial Applications*. InTech; Croatia, 2012. pp.5–26. ISBN: 978-953-51-0298-4
- [48] Ibekwe NN, Ameh SJ. Hyphenated techniques in liquid chromatography as current trends in natural products analysis. *International Research Journal of Pure & Applied Chemistry*. 2015;**7**(3):132-149
- [49] Wijesekera ROB. *The Medicinal Plant Industry*. CRC Press; Taylor & Francis Inc, Bosa Roca, USA. 1991. ISBN 9780849366697
- [50] Vlietinck AJ, Apers S. Biological screening methods in the search for pharmacologically active natural products. In: Tringali C, editor. *Bioactive Compounds from Natural*

Sources Isolation, Characterisation and Biological Properties. London: Taylor & Francis; 2001. ISBN 0-203-26972-1;

- [51] Rior RLP, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*. 2005;**53**(10):4290–4302
- [52] Sochor J, Dobes J, Krystofova O, Ruttkay-Nedecky B, Babula P, Pohanka M, Jurikova T, Zitka O, Adam V, Klejdus B, Kizek R. Electrochemistry as a tool for studying antioxidant properties. *International Journal of Electrochemical Science*. 2013;**8**:8464–8489
- [53] Taiga A. Quantitative phytochemical properties of mistletoe (*Viscum album*) from five different plants. *Research Journal of Agricultural and Environmental Management*. 2013;**2**(6):150–153
- [54] Frazer Sir JG. *The Golden Bough*. 3rd ed. London: Macmillan and Co, Limited St. Martin's Street; 1920
- [55] Chernyshov VP, Heusser P, Omelchenko LI, Chernyshova LI, Vodyanik MA, Vykhovanets EV, Galazyuk LV, Pochinok TV, Gaiday NV, Gumenyuk ME, Zelinsky GM, Schaefermeyer H, Schaefermeyer G. Immunomodulatory and clinical effects of *Viscum album* (Iscador M and Iscador P) in children with recurrent respiratory infections as a result of the Chernobyl nuclear accident. *American Journal of Therapeutics*. 2000;**7**(3):195–203
- [56] Twardziok M. Mechanism of action of *Viscum album* L. extracts in Ewing sarcoma [PhD thesis]. Freie Universität Berlin; 2015
- [57] Vicas SI, Rugina D, Socaciu C. Antioxidant activity of European mistletoe (*Viscum album*). In: Rao V, ed. *Phytochemicals as Nutraceuticals-Global Approaches to Their Role in Nutrition and Health*. InTech, Croatia; 2012. pp.115–134. ISBN 978-953-51-02038
- [58] Hajto T, Fodor K, Perjesi P, Nemeth Peter. Difficulties and perspectives of immunomodulatory therapy with mistletoe lectins and standardized mistletoe extracts in evidence-based medicine. *Evidence-Based Complementary and Alternative Medicine*. 2011;**6**:Article ID 298972. Hindawi Publishing Corporation
- [59] Gray AM, Flatt PR. Insulin-secreting activity of the traditional antidiabetic plant *Viscum album* (mistletoe). *The Journal of Endocrinology*. 1999;**160**(3):409–414
- [60] Adeeyo AO, Adefule AK, Ofusori DA, Aderinola AA, Caxton-Martins EA. Antihyperglycemic effects of aqueous leaf extracts of mistletoe and *Moringa oleifera* in streptozotocin-induced diabetes Wistar rats. *Diabetologia Croatica*. 2013;**42**(3):81–88
- [61] Marvibaigi M, Supriyanto E, Amini N, Adibah F, Majid A, Jaganathan SK. Preclinical and clinical effects of mistletoe against breast cancer. *BioMed Research International*. 2014;**15**:Article ID 785479
- [62] Kuttan G, Vasudevan DM, Kuttan R. Effect of a preparation from *Viscum album* on tumor development in vitro and in mice. *Journal of Ethnopharmacology*. 1990;**29**(1):35–41

- [63] Valentiner U, Pfüller U, Baum C, Schumacher U. The cytotoxic effect of mistletoe lectins I, II and III on sensitive and multidrug resistant human colon cancer cell lines in vitro. *Toxicology*. 2002;**171**(2-3):187–199
- [64] Antony S, Kuttan R, Kuttan G. Role of natural killer cells in iscador mediated inhibition of metastasis by adoptive immunotherapy. *Immunological Investigations*. 2000;**29**(3):219–231
- [65] Eno AE, Ibokette UE, Ofem OE, Unoh FB, Nkanu E, Azah N, Ibu JO. The effects of a Nigerian specie of *Viscum album* (Mistletoe) leaf extract on the blood pressure of normotensive and Doca-induced hypertensive rats. *Nigerian Journal of Physiological Sciences*. 2004;**19**(1-2):33–38
- [66] Li Y, Zhao YL, Yang YP, Li XL. Chemical constituents of *Viscum album* var. *Meridianum*. *Biochemical Systematics and Ecology*. 2011;**39**:849–852
- [67] Luczkiewicz M, Cisowski W, Kaiser P, Ochocka R, Piotrowski A. Comparative analysis of phenolic acids in mistletoe plants from various hosts. *Acta Poloniae Pharmaceutica-Drug Research*. 2001;**58**(5):373–379. ISSN 0001-6837
- [68] Poojary B, Belagali SL. Synthesis, characterisation and biological evaluation of cyclic peptides: Viscumamide, yunnanin A and evolidine. *Zeitschrift fur naturforschung section b-a journal of chemical sciences*, 2005;**60**(12):1313–1320
- [69] Larsson S. Mistletoes and thionins as selection models in natural products drug discovery.. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy* 49. Uppsala: Acta Universitatis Upsaliensis; 2004. 65pp. ISBN 978-91-554-6824-8;
- [70] Guzmán-Rodríguez JJ, Ochoa-Zarzosa A, López-Gómez R, López-Meza JE. Plant antimicrobial peptides as potential anticancer agents. *BioMed Research International*. 2015;**11**:Article ID 735087
- [71] Colegate SM, Molyneux RJ. *Bioactive Natural Products—Detection, Isolation, and Structural Determination*. 2nd ed. Taylor & Francis Group, LLC, USA; 2008
- [72] Simard EP, Engels EA. Cancer as a cause of death among people with AIDS in the United States. *Clinical Infectious Diseases*. 2010;**51**(8):957–962
- [73] Tantry MA. Plant natural products and drugs: a comprehensive study. *Asian Journal of Traditional Medicines*. 2009;**4**(6):241–249
- [74] Giudici M, Pascual R, de la Canal L, Pfüller K, Pfüller U, Villalain J. Interaction of viscotoxins A and B with membrane model systems: Implications to their mechanism of action. *Biophysical Journal*. 2003;**85**:971–981
- [75] Hemmateenejad B, Javidnia K, Nematollahi M, Elyasi M. QSAR studies on the antiviral compounds of natural origin. *Journal of the Iranian Chemical Society*. 2009;**6**(2):420–435
- [76] Kuramochi K. Synthetic and structure-activity relationship studies on bioactive natural products. *Bioscience, Biotechnology, and Biochemistry*. 2013;**77**(3):446–454