Chapter 10

Bioactive Molecules Profile from Natural Compounds

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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 derivates: 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 *officinali* 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,* Clemantis *vitalba L.,* Potentilla *reptans L.,* Lappa *maior Gartn.,* 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 phytocompounds, 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**.

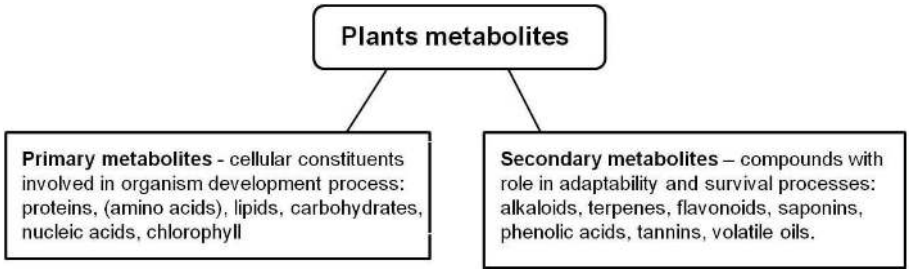

Figure 1. Plant metabolites.

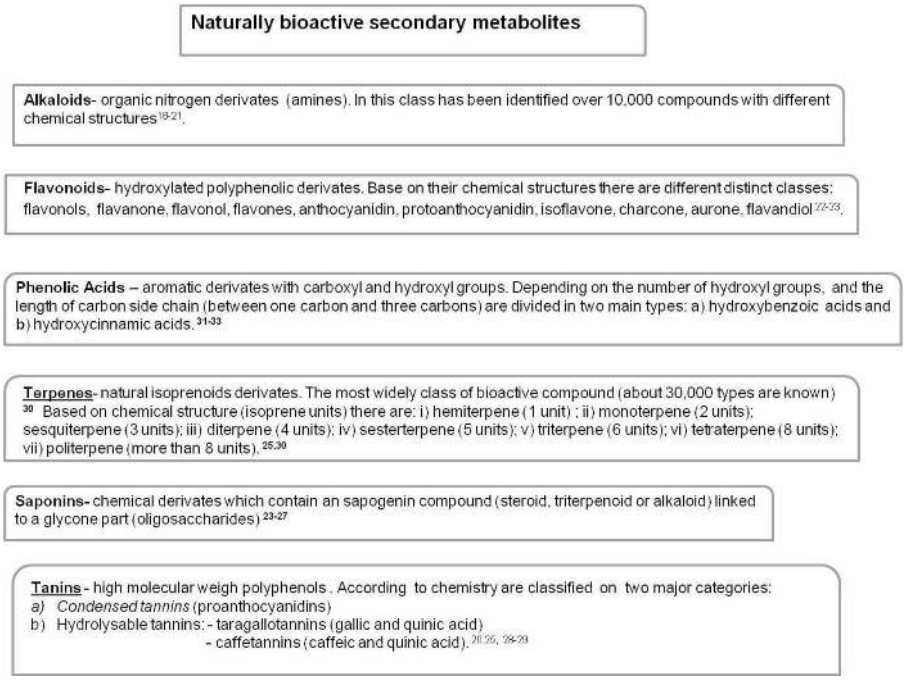

Figure 2. Schematic representation of plant bioactive compounds.

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, 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, serotonine, vinblastine, vincristine, brucine, capsaicin, solanine, tomatine, choline, etc.	[15, 21, 34]
Terpenes	*Hemiterpene*: isoprene, isovaleric acid	[15, 34]
	Monoterpene: limonele, eucalyptol, menthol, nerol, citral	
	Sesquiterpene: zinziberene, farnesol	
	Diterpene: cafestol, retinal, retinol	
	Sesterterpenes: bulgarene, farnesol, lindarene	
	Triterpene: provitamin A, betulin, cymarin	
	Tetraterpene: lycopen, α si β carotenoids	
	Polyterpene: vitamin E, gutta-percha	
Flavonoids	*Flavones:* luteolin, diosmetin, apigenin	[15, 22, 23]
	Flavonols: quercetin, myricetin, rutin, kaempferol	
	Flavanones: hesperetin, naringenin	
	Flavanonol: silymarin, taxifolin	
	Isoflavones: daidzin, genistin	
	Anthocyanidin: cyanidin, delphinidin, peonidin, petunidin	

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].

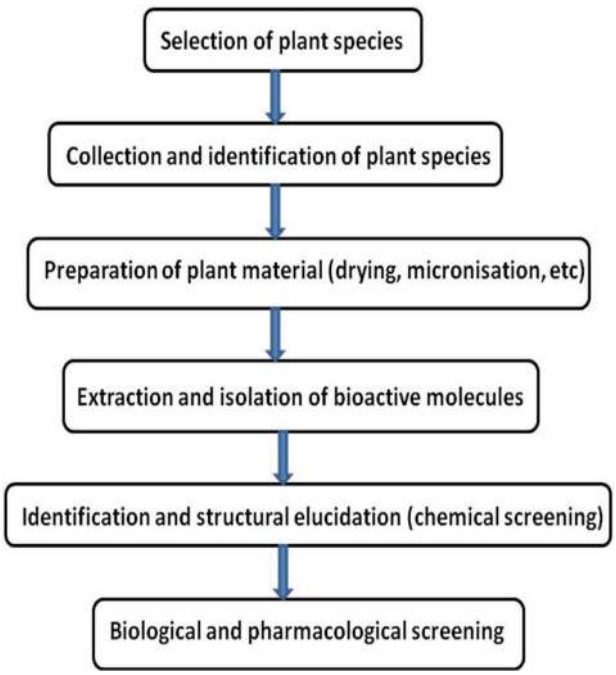

Figure 3. Flowchart of plant bioactive molecules profiling.

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 authentification 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)
	Nuclear Magnetic Resonance **(NMR) spectroscopy:**
	(a) *One-dimensional techniques:* ^{1}HNMR, ^{13}CNMR, ^{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-lr, 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
Chromatography methods	**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
	Liquid chromatography: LC/UV; LC/MS; LC/UV/MS; LC/MS-MS; LC/NMR, LC-UV-DAD, HPLC-NMR
Other analytic techniques	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, cardiotonic, 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)**.

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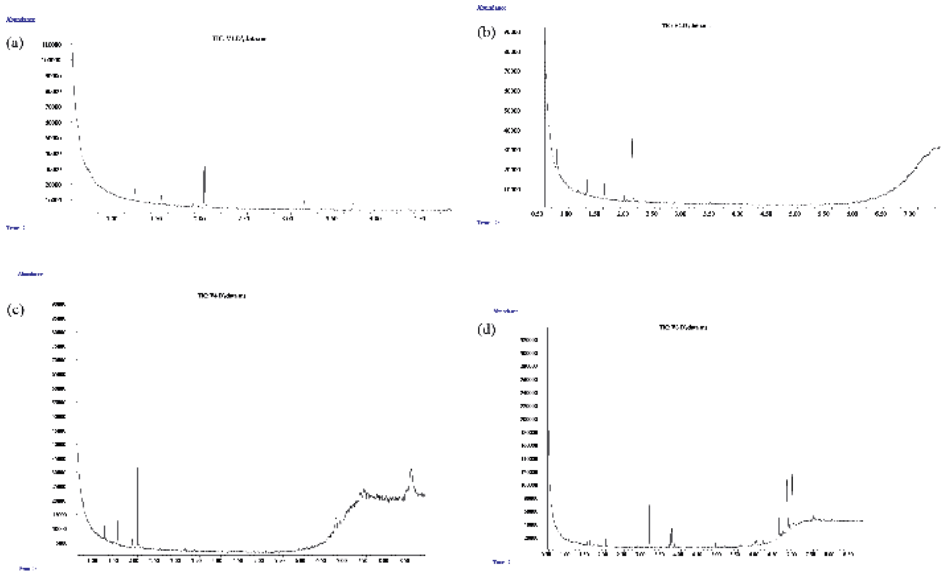

Figure 4. TIC of (a) V_1 extract, (b) V_2 extract, (c) V_3 extract and (d) V_4 extract.

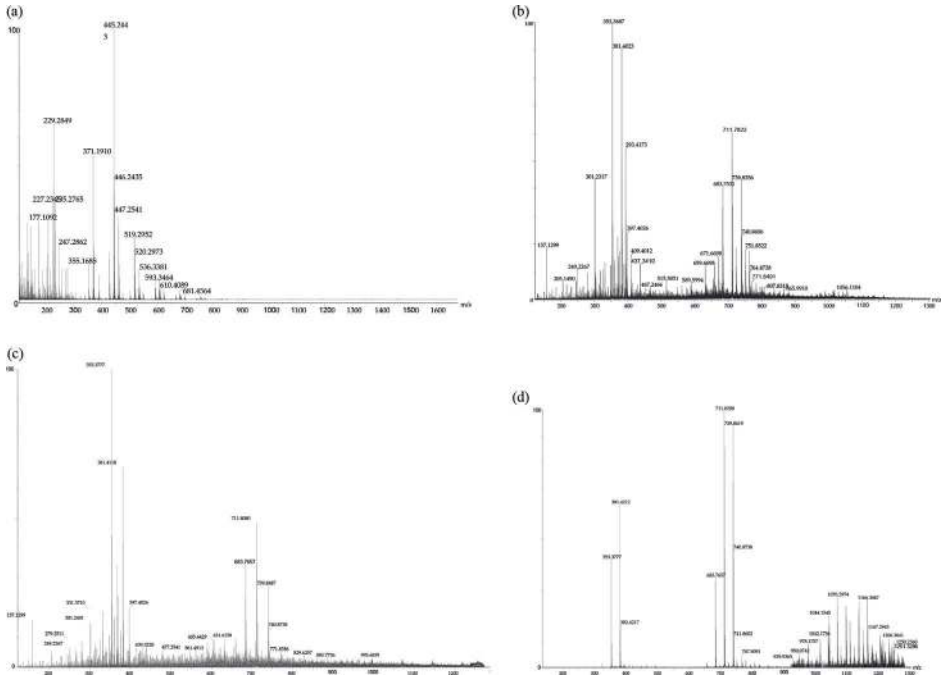

Figure 5. Positive ion mode TOF-MS of (a) V_1extract, (b) V_2extract, (c) V_3extract and (d) V_4extract.

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) 3330–3130 cm^{-1} (NH_3+ groups); (iii) symmetric absorption at 2080–2140 cm^{-1}or 2530–2760 cm^{-1}; (iv) 1500–1600 cm^{-1} (ammonium group deformation vibrations); (v) 1610–1660 cm^{-1} (carboxylate group); (vi) 1724–1754 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.

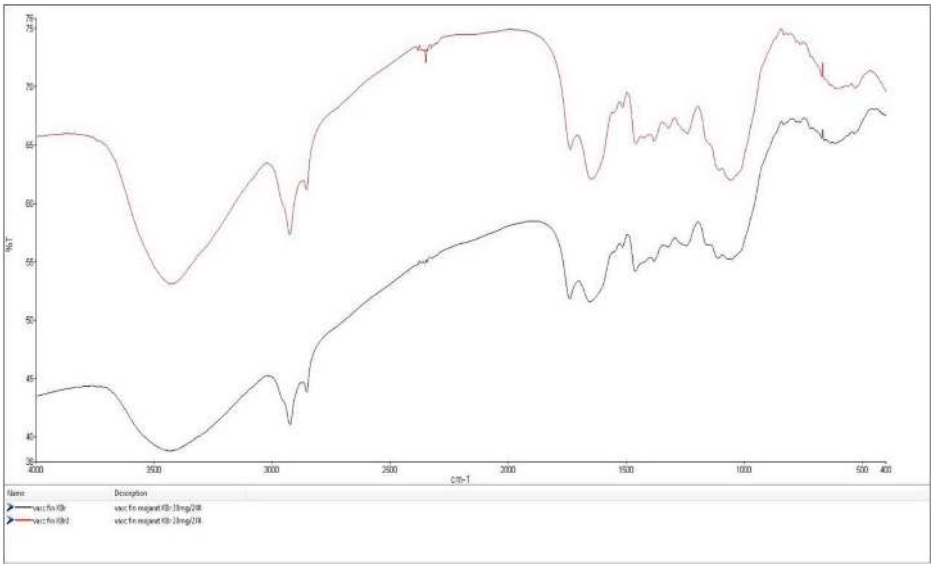

Figure 6. The FT-IR spectra for the mistletoe sample.

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.

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