ULTRASOUND EXTRACTION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS OBTAINED FROM DIFFERENT ORGANS OF *DATURA INNOXIA*

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In this work, a green technique for bioactive compounds extraction from different vegetative organs (leaves, flowers, seeds, stem and root) of *Datura innoxia*, assisted by ultrasound (UAE), was performed using ethanol and 1-butanol as solvents. The chemical compositions of the extracts and quantitative determination of alkaloids (scopolamine) were investigated using different methods: Fourier transform infrared (FTIR) spectroscopy, fluorescence spectrophotometry (FL), ultraviolet-visible (UV-VIS) spectroscopy and gas chromatography with flame ionization detection (GC-FID). Based on the effect of solvent polarity on extraction, the results indicated that 1-butanol extracts show high extraction efficiency of lipophilic compounds in a non-polar solvent. Moreover, the results show a high abundance of the main classes of secondary metabolites (terpenes, alkaloids, phenolics), and alkanes, esters, ketones, alcohols, carbohydrates and polysaccharides (lignin and cellulose) were among the classes of compounds identified in matrix extracts. Taking into account the mentioned aspects, this study demonstrated that UAE can be adopted to obtain secondary metabolites with active principles of pharmacological and cosmetic importance from *Datura innoxia* dry biomass for sustainable biorefining.

Keywords: biomass, biorefinery, green extraction, chromatography, spectrophotometry, spectroscopy, secondary metabolites

INTRODUCTION

Bioactive compounds refer to molecules that have some biological activity and, in plants, are secondary metabolites that can have pharmacological or toxicological effects in humans and animals.[1] They can be obtained by separation and purification, by applying both conventional and non-conventional extraction techniques, and can be identified using chromatographic, spectroscopic and spectrophotometric characterization methods.

All plants produce secondary metabolites and usually they can store several major compounds, from different structural classes and through biochemical pathways, which are usually accompanied by dozens of minor components. There are four major biosynthetic pathways for the production of these bioactive secondary metabolites: 1) shikimic acid pathway, 2) malonic acid pathway, 3) mevalonic acid pathway, and 4) non-mevalonate (MEP) pathway.[2] It is representative to find a complex mixture, which differs from one plant component to another, sometimes among individual plants and usually among species.[3,4] Within a single plant species, between 5000 and 20000 individual primary and secondary metabolites can be produced.[3-5]

There are two categories of metabolites in biomass, which is any organic material that can constitute the main raw material of a biorefinery: primary and secondary. Primary metabolites, which are chemicals directly involved in plant development or reproduction, include macromolecular compounds, such as polysaccharides (lignin and cellulose), amino

acids, proteins and lipids. Secondary metabolites (low content), which are derived by biosynthesis from primary plant metabolites and are not directly involved in the development or reproduction of plants, allowing them to interact with the environment, are classified into three main classes, namely 1) alkaloids, 2) terpenes and 3) phenolic compounds. Due to their functionality and biological activity, secondary metabolites are highly relevant in biorefineries.[6,7]

The conversion of biomass resources to bioactive compounds by various techniques, especially ultrasound assisted extraction (UAE) is thus a very demanding and trending research topic. In the last 20 years, we have witnessed an amazing growth in the application of ultrasound as a source of energy in various fields of science, especially in the field of chemistry. The number of articles devoted to almost all types of analysis dealing with the use of ultrasound as an energy source continues to grow from year to year.[8] The power of ultrasound is considered a green technology in achieving the goal for chemical engineering and sustainable ecological extraction. It is well known that ultrasound has a significant effect on the growth rate of various processes in the chemical and food industries. Solid-liquid solvent extraction is an operation found in many industrial processes.[9,10]

Using ultrasound, complete extractions can now be carried out in minutes with high reproducibility, reducing solvent consumption, simplifying handling and processing, providing higher end product purity and consuming only a fraction of fossil energy.[11] Several classes of chemical compounds, such as flavours, pigments, antioxidants, and other organic and mineral compounds, have been efficiently extracted and analysed from a variety of sources (mainly animal tissues, microalgae, yeasts, foods, and plant materials).[12-16]

Various studies have demonstrated that no single solvent can extract all compounds present in plant raw material, indicating that solvent polarity significantly affects the extracts.[12-16] In general, the selection of the solvent should be in accordance with the chemical nature of the target compounds. For example, polar solvents (ethanol, methanol, ethyl acetate, *etc*.) are used for the extraction of hydrophilic compounds from plants, while nonpolar solvents (1-butanol, hexane, ether, petroleum ether, *etc*.) are preferred for the extraction of lipophilic secondary metabolites.[17,18]

Datura innoxia, an indigenous plant from America and Asia, known in Romania under the improper name “angel’s trumpet”, is part of the Solanaceae plant family and is widespread in Europe, especially as a medicinal and ornamental plant. In terms of chemical composition, it is recognized for its rich content of tropane alkaloids, especially scopolamine. The occurrence of alkaloids in all plant organs is of crucial importance from the point of view of their use in medicine, but also as a risk of toxicity for humans and animals, being on the border between beneficial and harmful. *Datura innoxia* is recognized for its importance as a source of drugs in medicine and for its pharmacological properties.[19,20] Moreover, in addition to its role as a plant with a beneficial, harmful or neutral effect, studies in the specialized literature present the *Datura innoxia* species as an invasive plant species that can be accidentally or deliberately introduced, and has the ability to acclimatize to new environmental conditions.[21]

Taking into account previous findings, this paper presents the effect of solvent polarity on the extraction efficiency of secondary metabolites. Qualitative and quantitative analysis results will contribute to a better understanding of the methods for characterizing the main metabolites extracted in a solid-liquid system, by UAE, from the plant biomass formed by the vegetative organs of *Datura innoxia*. This approach is in line with sustainable biorefining, allowing the valorisation of bioactive compounds for use in the pharmaceutical and cosmetic domains.

EXPERIMENTAL

Plant material and chemicals

Datura innoxia plant organs (leaves, flowers, seeds, stem and root) were collected from a local area, Romania (4523005.7” N, 2702059.6” E, altitude of 118 m) at maturity. In taxonomic terms, the plant belongs to the order Solanales, family Solanaceae, genus *Datura*, species *D. innoxia*. It is a perennial, dicotyledonous plant, known in Romania under the improper name of “angel's trumpet”.[22] It grows in the form of bushes, reaching a height of 90-200 cm,[23-25] and is spread throughout the world in warm climate areas.[26] From the morphological point of view, the plant was identified according to reviewed literature.[27] The dark green leaves have an asymmetrical base and a length of 20-30 cm. The large white flowers range from 20 cm to 30 cm in length and bloom at sunset. About 200 kidney-shaped seeds are stored in the cavity inside the capsule divided into several chambers (lojas).[28-30] The stem, aerial vegetative organ, green in color with a smooth

appearance, has a cylindrical shape that bifurcates into two secondary branches. The root is pivoting and consists of a primary root, from which thin secondary roots develop.[25]

Reagents and standards as hyoscine standard (≥99%) were purchased from Sigma Aldrich, ethanol (HPLC analytical grade), the solvent used for calibration was purchased from Sigma Aldrich (Darmstadt, Germany), and ethanol (96%), 1-butanol (99.6%) solvents, used for the ultrasonication process, were purchased from Chemical Company, Romania. Solvent selection was performed according to characteristic properties (polarity, boiling point, dielectric constant and density).

Moisture content determination of plant material

The fresh vegetative organs of the plant were manually removed and the fruits were peeled by hand to separate the capsule from the seeds. The collected plant biomass was transported to the laboratory and kept at room temperature. In general, before extraction, the plants can be dried at room temperature under controlled conditions to prevent any deterioration of the chemical composition.[31]

The moisture content of the plant material was determined using the drying method according to Rozmarin *et al*.[32] The plant material was pre-dried in an oven at a temperature of 100 °C for 3 hours and then placed in a desiccator for 15 minutes. The crucibles were subjected to drying and then weighed repeatedly at smaller intervals (1 hour) until constant mass was finally reached. The moisture content was determined using the relation:

$$U\% = \frac{m_2 - m_3}{m_2 - m_1} \times 100 \quad (1)$$

where U – relative humidity, m_1 – mass of empty filter crucible (g), m_2 – mass of filter crucible with wet material (g), m_3 – mass of filter crucible with dry material (g).

Ultrasound-assisted extraction (UAE)

Samples (0.2 g) of dry *Datura innoxia* plant biomass, in powder form, taken individually for each vegetative organ (leaves, flowers, seeds, stem and root), were weighed on a Precisa XT 120A analytical balance, with an accuracy of 10^{-4} g. For each vegetative organ, 3 samples were processed to ensure the repeatability and reproducibility of the method. The weighed sample mass was placed in a capped microtube (2 mL), over which 2 mL of solvent was added. For the ultrasound procedure, 2 solvents were used: 1) ethanol; and 2) 1-butanol. After the solvent was added, the samples were left to macerate for 24 h.

After the maceration process, the samples were subjected to the ultrasound assisted extraction process for 45 minutes, at a temperature of 21 °C, using an experimental installation (Bandelin Sonorex ultrasound bath (P = 80/320W; f = 35kHz). After ultrasonication, the samples were centrifuged at 15000 rpm for 3 minutes, using a Hermle Z 229 centrifuge. The centrifugation step was followed by the supernatant transfer step, the extract was placed in a clean microtube with a cover (2 mL), after it was previously filtered through Iso-Disc filters, with a porosity of 0.45 μm, made of poly-tetra-fluor-ethylene (PTFE). Both the extracts generated and the residues obtained from the processing of the plant material were kept in the refrigerator until the analysis of all the samples was completed.

Characterization methods

Gas chromatography with flame ionization detection (GC-FID)

Analysis was performed by manual injection using the Hewlett Packard HP 5890 Series II Gas Chromatograph (GC) system, U.S.A. For the extracts analysis and scopolamine standard, the optimized working conditions of the equipment were the following: 1) nitrogen as mobile phase from the hydrogen generator (HG 2200) + nitrogen-air generator (ANG 2381) CLAIND model; 2) SPB-1 Supelco capillary column (30 m long, 0.32 mm internal diameter and 0.25 μm liquid film thickness inside the column) as stationary phase; 3) 280 °C injector operating temperature and 250 °C detector operating temperature; 4) 10 μL injected volume under splitless conditions of 1:100 (three replicates were performed); 5) temperature gradient was as follows: 100 °C for 1 min, ramp at 15 °C/min to 180 °C (constant 5 min), followed by ramp at 5 °C/min to 300 °C (constant 20 min). The chromatograms revealed the profile of the dependence between the area of the chromatographic peak associated with scopolamine versus the retention time, $t_R \approx 12$ min.

UV-Vis spectrophotometry

All absorption spectra were recorded using a V-550 (Jasco, Japan) UV-Vis spectrophotometer. The absorbance and the wavelength of the peaks recorded both for the ethanol and 1-butanol extracts obtained from the plant organs and for the hyoscine standard were determined by scanning in the wavelength range between 190 and 900 nm at room temperature. Four determinations were performed for each sample.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra were recorded on a Varian Digilab FTS 2000 Scimitar FTIR Spectrometer, U.S.A. in the attenuated total reflection (ATR) mode with horizontal plate that allows direct application of liquid samples. A zinc selenide (ZnSe) crystal plate at 45° was used to sample the liquid on the top of the plate. Data were collected in absorbance mode and in the wavelength range from 4000 to 400 cm^{-1}. A spectral resolution of 4 cm^{-1} was used. Based on the value of the peaks in the IR region

and compared to that previously reported, detection was performed.

Fluorescence spectroscopy

Fluorescence spectra were recorded using a Perkin Elmer LS 50B Fluorescence Spectrophotometer, U.K., and determined in pre-scan mode. For the measurement of the excitation spectrum, the wavelength was fixed at 280 nm and the emission wavelength was from 200 (slits fixed at 5 nm) to 700 nm (slits fixed at 10 nm). The number of spectra accumulations was 3 and the scan speed of 500 nm/mm. For the quantitative determination of scopolamine, a stock solution of hyoscine (800 mg/L) in ethanol was prepared. The fluorescence spectra of the hyoscine standard solution were recorded under the same conditions.

Standardization of analysis methods for hyoscine

Quantification was carried out according to the characterization method using hyoscine as external standard method by means of a five-points calibration curve. The parameters are listed in Table 1.

Table 1
Summary of the parameters of the calibration curves for hyoscine (scopolamine) according to the characterization method

Hyoscine standard	UV-Vis	FL	GC-FID
Linear regression	y *= 0.031x + 0.047	y * = 14.136x + 29.863	y *= 0.015x – 0.343
N	7	4	5
Linear correlation coefficient (r^2)	0.993	0.919	0.999
Standard deviation of the slope (σ)	0.004	6.904	0.931

*y = ax + b, where x is the concentration of the compound, y = absorbance (UV-VIS), intensity (fluorescence spectroscopy) and peak area (GC-FID)

Statistical analysis

Standard deviation parameters were calculated applying the following formula:

$$\sigma = \sqrt{\sum \frac{(X_i - \mu)^2}{N}} \quad (2)$$

where σ = population standard deviation; μ = population mean; X_i = element from the population; N = number of elements in the population. Concentrations are presented as the mean of analyses for 3 replicates, accompanied by 2 × standard deviation of the measurements made, with a 95% confidence interval. The mean – standard deviation – of three replicates was used to report all data.

RESULTS AND DISCUSSION

Spectroscopic analysis

Non-chromatographic techniques are extremely valuable for the identification, isolation and characterization of bioactive compounds from plant biomass extracts. The use of UV-vis, FL and FTIR spectroscopic techniques can provide information for identifying compounds of interest in plants, both qualitatively and quantitatively, in a less time-consuming, simple, fast and reliable way.[17,33-35]

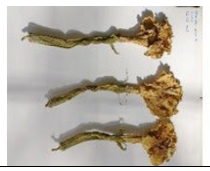
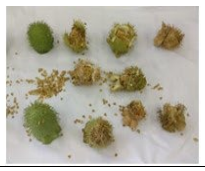
Therefore, in the present study, UV-vis, FL and FTIR techniques are employed to analyse the chemical structure of the extracts of *Datura innoxia* dry biomass (leaves, flowers, seeds, stem and root), in the two solvents used – ethanol (EtOH) and 1-butanol (1-BuOH) – in order to examine both qualitatively and quantitatively, the secondary metabolites for tropane alkaloid scopolamine.

UV-vis analysis of the extracts led to a complete characterization that showed a varied content of phytochemical compounds. For each vegetative organ of the *Datura innoxia* plant (leaves, flowers, seeds, stem and root), the whole spectrophotometric fingerprinting profile of the UV-vis absorption spectra in the EtOH and 1-BuOH extracts was presented in Figure 1 (a and b). The UV-vis characterization of the extracts obtained with the two solvents used is presented in Table 2. In this table, it can be observed that scopolamine was identified in all vegetative organs at λ = 206 nm.

Spectrophotometric peaks attributed to specific phytochemical compounds can be observed, for example, a peak at 665 nm, corresponding to chlorophyll and its derivatives – chlorophyll being present in the extracts of leaves (EtOH and 1-BuOH), flowers (1-BuOH) and stem (1-BuOH).[36,37] The peaks at 410 nm (EtOH) and 428, 479 nm (1-BuOH) for the extracts from leaves, and the peak at 420, 466 nm (1-BuOH) in the extract from flowers, respectively, were correlated with a variety of carotenoids and pheophytin. An important correlation was observed especially in the spectral region between 223 nm and 661 nm,

attributed to phenolic compounds and flavonoids,[37,38] observed in all plant organs extracted in EtOH and 1-BuOH. Regarding the percentage composition (%) related to the total area of the peaks in the spectra (Fig. 2 (a and b)), we can observe the presence of eight classes of secondary metabolites, of which six could be identified: alkaloids (scopolamine), terpenes (saponins), phenolic compounds (tannins and flavonoids), pigments (carotenoids and chlorophyll).

Table 2
UV-vis characterization of *Datura innoxia* extracts obtained with the two solvents

	Leaves		Flowers		Seeds		Stem		Root	
D. innoxia										
Solvents	EtOH	1-BuOH	EtOH	1-BuOH	EtOH	1-BuOH	EtOH	1-BuOH	EtOH	1-BuOH
Peaks λ (nm)	210 328 410 665	209 222 428 479 616 666	202 217 280 328	209 223 286 318 420 466 666	203 278	203 278	211 197	211 221 661	210 281 781	218 284 330 891

Notes: the absorption band between 202 and 218 nm, attributed to the presence of scopolamine (biomass of leaves, flowers, seeds, stem and root); the absorption band between 219 nm and 224 nm, attributed to phenols (leaf, flower and stem biomass); the absorption band between 278 and 289 nm, attributed to the presence of tannins (biomass of leaves, flowers, seeds, stem and root); the absorption band in the range of 315–400 nm, attributed to the presence of flavonoids (leaf, flower, stem and root biomass); the absorption band in the range of 400–539 nm, attributed to the presence of carotenoids and pheophytin (leaf and flower biomass); the absorption band between 665 and 668 nm, attributed to the presence of chlorophyll (leaf, flower and stem biomass).

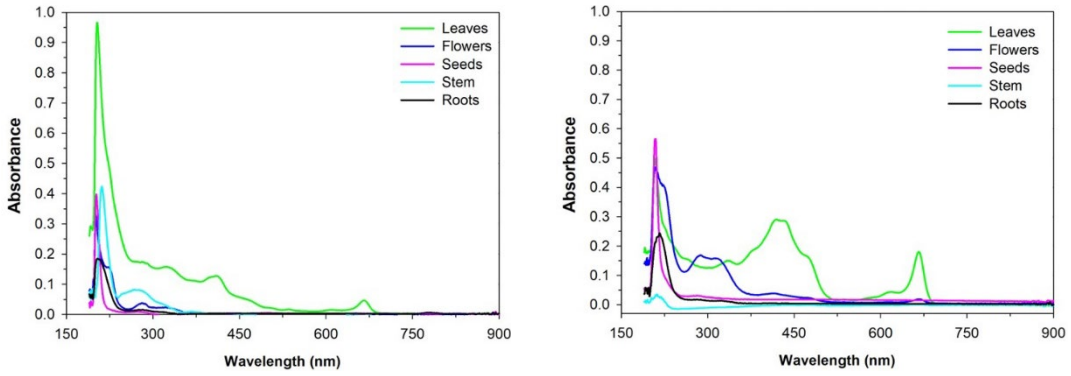


Figure 1: UV-vis spectra of the extracts of *Datura innoxia* leaves, flowers, seeds, stems and roots in EtOH (a) and 1-BuOH (b)

The results obtained are consistent with the degree of lipophilicity of scopolamine, but also of other compounds, and, therefore, a high extraction efficiency is obtained using a non-polar solvent, in our case, 1-BuOH (Fig. 2 (b)).[39,40]

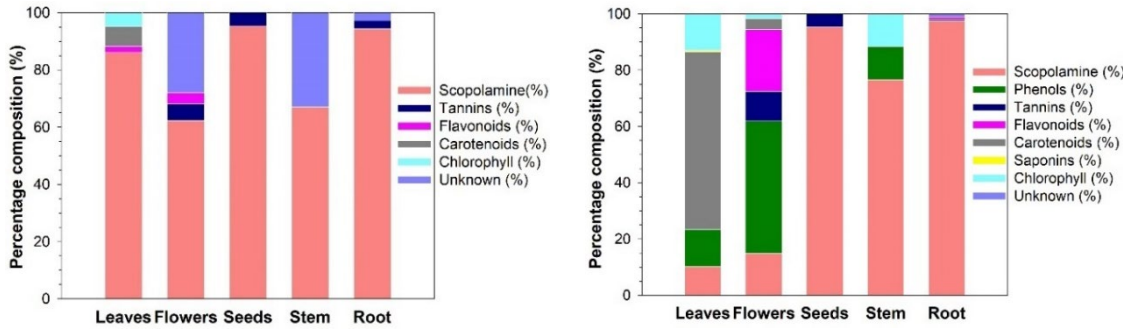
Regarding the influence of solvents on the distribution of scopolamine, it varied in the following order:

- for EtOH leaves > stem > seeds > flowers > root;
- for 1-BuOH seeds > leaves > flowers > root > stem.

Regarding the amount of scopolamine (Fig. 3), the highest content was found in the leaf extracts for EtOH (297.19 ± 0.01 mg/kg) and in the seed extracts for 1-BuOH (173.33 ± 0 .02 mg/kg), and

the lowest content was determined in the root extract for EtOH (45.89 ± 0.01 mg/kg) and in the stem extract for 1-BuOH (12.08 ± 0.01 mg/kg).

The obtained results present clear evidence that scopolamine alkaloid has variable concentrations from one vegetative organ to another, depending on the stages of plant development (collected at maturity), as has been suggested previously in the literature,[41] but also the fact that a large number of environmental factors can influence this content (light and ultraviolet radiation, heat stress, drought stress, and soil salinity stress).[42,43]



(a) (b)

Figure 2: Percentage composition of metabolites in EtOH (a) and 1-BuOH (b) *Datura innoxia* extracts related to the total area of the peaks in the UV-vis spectra

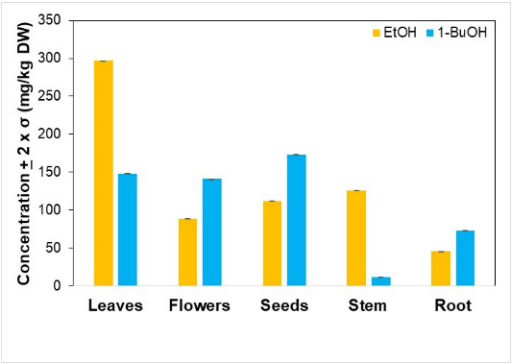


Figure 3: Scopolamine concentration (mg/kg dry weight) obtained in EtOH and 1-BuOH extracts of *Datura innoxia* (error bars given as ± 2x standard deviation for 3 replicate measurements at the 95% confidence interval)

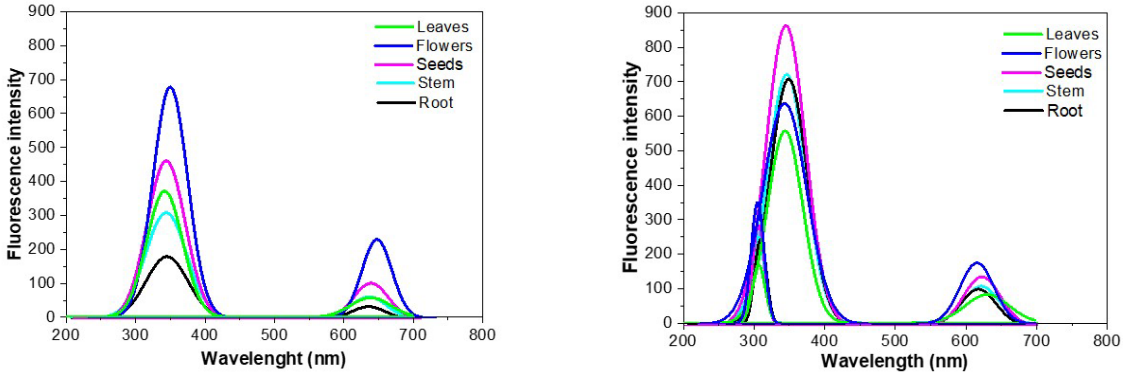
In addition to UV-vis, FL is a reliable and sensitive method for detection of secondary metabolites from plant biomass. Figure 4 (a and b) shows the deconvoluted fluorescence spectra of the *Datura innoxia* biomass extracts for each vegetative organ separately (leaves, flowers, seeds, stem and root) in EtOH (Fig. 4(a)) and 1-BuOH extracts (Fig. 4(b)), respectively. The spectra are characterized by peaks attributed to tocol derivatives (λ_{em} = 308 nm),[37,44,45] scopolamine (λ_{em} = 345 nm), the pheophytin compound and chlorophyll derivatives (λ_{em} = 620 nm),[45] with a dependence of fluorescence on the nature of the solvent (1-BuOH > EtOH).

Also, from Figure 4 (a and b), it can be remarked that in almost all spectral fields, the difference is made by the appearance of the peak at λ_{em} = 308 nm, attributed to tocol derivatives in leaf extracts in EtOH (Fig. 4(a)), and in the extracts of leaves, flowers, seeds, stems and roots in 1-BuOH (Fig. 4(b)). This correlation may appear as a result of the change in the concentrations of bioactive compounds in the sample plant tissue that exhibit fluorescence, but also as a result of the fact that non-polar solvents are used to solubilize mostly lipophilic compounds.[37,46]

Regarding the highest percentage composition (%) related to the total peak area in the spectrum, represented in Figure 5 (a and b), the following content of scopolamine was determined: 85% in leaves, 77% in flowers, 83% in seed, 86% in stems, 86% in roots for the EtOH extracts, and 78% in leaves, 72% in flowers, 79% in seeds, 80% in stems, 80% in roots for the 1-BuOH extracts. These results confirm the affinity of scopolamine for the nonpolar solvent, which may be in agreement with the lipophilicity of the compound. As regards tocopherols and tocotrienols, following the determination of the percentage composition, they were identified only in the 1-BuOH extracts,

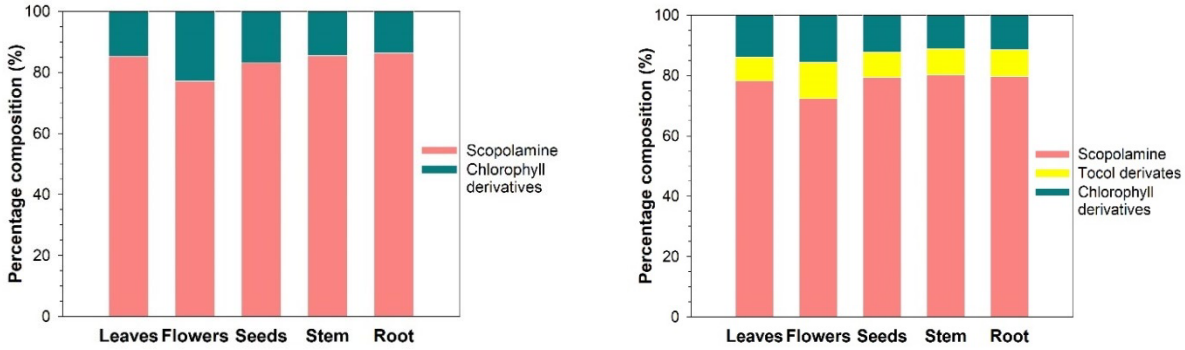
mainly in the flower extracts (12%). In the whole plant, the percentage composition was 46%, which is in agreement with literature data.[47]

Chlorophyll derivatives were also identified following the determination of the percentage composition in the extracts of all plant tissues both in EtOH (82%) (Fig. 5(a)) and in 1-BuOH (64%) (Fig. 5(b)).



(a) (b)

Figure 4: Fluorescence spectra of *Datura innoxia* leaf, flower, seed, stem and root extracts in EtOH (a) and 1-BuOH (b)



(a) (b)

Figure 5: Percentage composition of metabolites in *Datura innoxia* extracts in EtOH (a) and 1-BuOH (b), related to the total area of the peaks in the fluorescence spectrum

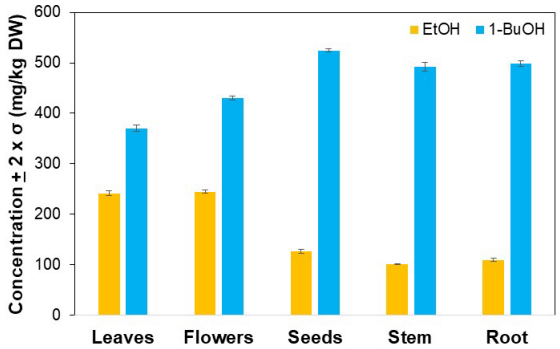


Figure 6: Scopolamine concentration expressed in mg/kg dry weight obtained in EtOH and 1-BuOH extracts of *Datura innoxia* plant biomass (error bars given as ± 2x standard deviation for 3 replicate measurements at the 95% confidence interval)

As for the tropane alkaloid scopolamine, it was identified in all the vegetative organs of the plant, and following quantitative analysis, it was determined that the highest content is in the flower extract in EtOH (244.56 ± 2.99 mg/kg) and in the 1-BuOH extracted seeds (524.59 ± 2.81 mg/kg), while the lowest content is in the EtOH-extracted stems (101.01 ± 0.57 mg/kg) and in 1-BuOH-

extracted leaves (370.09 ± 6.01 mg/kg). Figure 6 shows the concentration of scopolamine in *Datura innoxia* plant biomass extracts in EtOH and 1-BuOH solvents. It can be seen that the highest scopolamine content was found in seeds (524.59 mg/kg dry weight) extracted in 1-BuOH. Error is given as ± 2 x standard deviation for 4 replicates, with 95% confidence interval.

In conclusion, it can be mentioned that the chemical analysis by fluorescence spectroscopy can be considered a quick alternative for the quantitative determination of scopolamine in *Datura innoxia* plant biomass. Moreover, from a qualitative point of view, the percentage composition of each active ingredient in different plant tissues of *Datura innoxia* can be determined related to the total area of the peaks in the spectrum.

Fourier transform infrared (FTIR) spectroscopy is, in our opinion, the most powerful, rapid, non-destructive method to analyse the chemical structure of entire *Datura innoxia* material. Screening results for these phytochemical compounds showed that the EtOH and 1-BuOH extracts of plant biomass are enriched with constituents belonging to both the primary metabolite class (lipids, proteins and carbohydrates),[48] and the class of secondary metabolites (alkaloids, phenolic compounds, terpenes, tannins and saponins).[38] In this context, the possibility of determining the distribution of the scopolamine alkaloid in the plant *Datura innoxia* was described.

To show the presence of different functional groups and chemical compounds, such as primary and secondary metabolites, FTIR spectra were recorded, and the results are presented in Table 3, while the spectra obtained for each vegetative organ are shown in Figure 7 (EtOH) and Figure 8 (1-BuOH). The characteristic peaks of the IR spectra for biomass samples are in the range 1800–600 cm^{-1}.

The FTIR spectrum of the EtOH and 1-BuOH extracts showed the peak at 2926 cm^{-1} (EtOH) and 2932 cm^{-1} (1-BuOH), which may indicate the presence of the C-H aliphatic group, which can be associated with the absorption bands for methyl (CH_3), methylene (CH_2) and methine (CH) characteristic of the carboxylic acid.[49] The existence of the peak at 2926 cm^{-1} (EtOH) and 2932 cm^{-1} (1-BuOH) may indicate the presence of the C-H bond, which can be associated with the absorption bands for methyl (CH_3), methylene (CH_2) and methine (CH).[37,49]

The appearance of the absorption band at 1748 – 1700 cm^{-1} is due to the C=O aliphatic group associated with the ester carbonyl group. The intensity of the peak at 1656 cm^{-1} corresponds to band I of the amide group. The absorption of the peak at ~1701 cm^{-1} can indicates the presence of uronic acid in EtOH extracts of flowers and roots (Fig. 7).[49,50] At the same time, according to the specialized literature, the presence of the peak at 1705 cm^{-1} can also be associated with the group of fatty acids, such as oleic acid, which is also found only in flowers and roots extracted in EtOH (Fig. 7).[48,51]

Aldehydes of a saturated fatty acid were also identified in the seed (1-BuOH) (Fig. 8) and stem (EtOH) extracts (Fig. 7).[52] The intensity of the peak at 1656 cm^{-1}corresponds to band I of the amide group (carbonyl group) and band II of the amide group (CN stretching vibration (νCN) and NH bending vibration (δNH). Another characteristic peak in the ~1325 cm^{-1} absorption band identified in glycoproteins, which does not correspond to the stem extract in 1-BuOH (Fig. 8).[53,54] The peak at 1452 cm^{-1} is present in all EtOH-extracted plant biomass extracts (Fig. 7) and is characteristic of the absorption band for alcohols, C–OH attributed to bending vibration motions.[38,55] Another characteristic peak in the ~1325 cm^{-1} absorption band is CN stretching vibration (νCN) and NH bending vibration (δNH) bend associated with lignin due to the C–O chemical bond in the structure of the chemical compound syringyl, and which was identified in all EtOH extracts from the plant biomass (Fig. 7).[56] The peak recorded at ~ 1379 cm^{-1}, showing the C–H bond, can be associated with cellulose, hemicelluloses, and lignin, and is found in all dry biomass extracts (EtOH and 1-BuOH) (Fig. 7 and Fig. 8).[48,56] At the same time, in other literature studies, the absorption of the peak at 1375 cm^{-1} indicates the band for the C-H bond, specific to alkenes.[49] In *Datura innoxia* biomass, the peak absorption at 1379 cm^{-1} can be associated with the lycopene compound found in all extracts (EtOH and 1-BuOH) from all vegetative organs.[48]

In leaves, flowers, seeds, stems and roots extracted in 1-BuOH (Fig. 8), a characteristic peak was identified at 1249 cm^{-1} and 1252 cm^{-1}, respectively, with symmetrical C-O-C aromatic acid ester bonds and with stretching vibration of phenolic C–OH groups, which exhibits the characteristic absorptions for esters and eugenol in volatile oils.[55]

Table 3
Comparative chemical structure of *Datura innoxia* dry biomass extracts in EtOH and 1-BuOH obtained by ultrasound assisted extraction

Functional groups	Wavenumber (cm^{-1})	Leaves		Flowers		Seeds		Stem		Root	
		EtOH	1-BuOH	EtOH	1-BuOH	EtOH	1-BuOH	EtOH	1-BuOH	EtOH	1-BuOH
Alcohols, phenols, carbohydrates	3327 – 3700										
Lipids, methoxy derivatives, aldehydes	2800 – 2974										
Alkanes	2850 – 3000										
Aldehydes	2695 – 2830										
Fatty acid ester group and glycerides	1730 – 1775										
Fatty acid group - oleic acid	1700 – 1715										
Uronic acid	~ 1701										
Glycoproteins	~1659, 1549										
Aldehydes of a fatty acid	1656										
Alcohol	1452										
Aromatic compounds	1462										
Lignin	~ 1325										
Cellulose, hemicelluloses and lignin	~ 1379										
Lycopene	~ 1379										
Carboxylic acids	~ 1270										
Esters and eugenol in volatile oils	1249, 1252										
Ester group	1159, 1113										
Amines	1045, 1042										
Terpenes	< 1000										
Monoterpenoid of the phenolic type, carvachol	810										
Sesquiterpene, α-Bisabolol	~ 739										
Glycosides	~ 991										
Carbohydrates	900-953										
Tropane alkaloids (scopolamine)	847, 880										
Alkenes	652 – 685										

Notes: orange colour for EtOH and blue colour for 1-BuOH

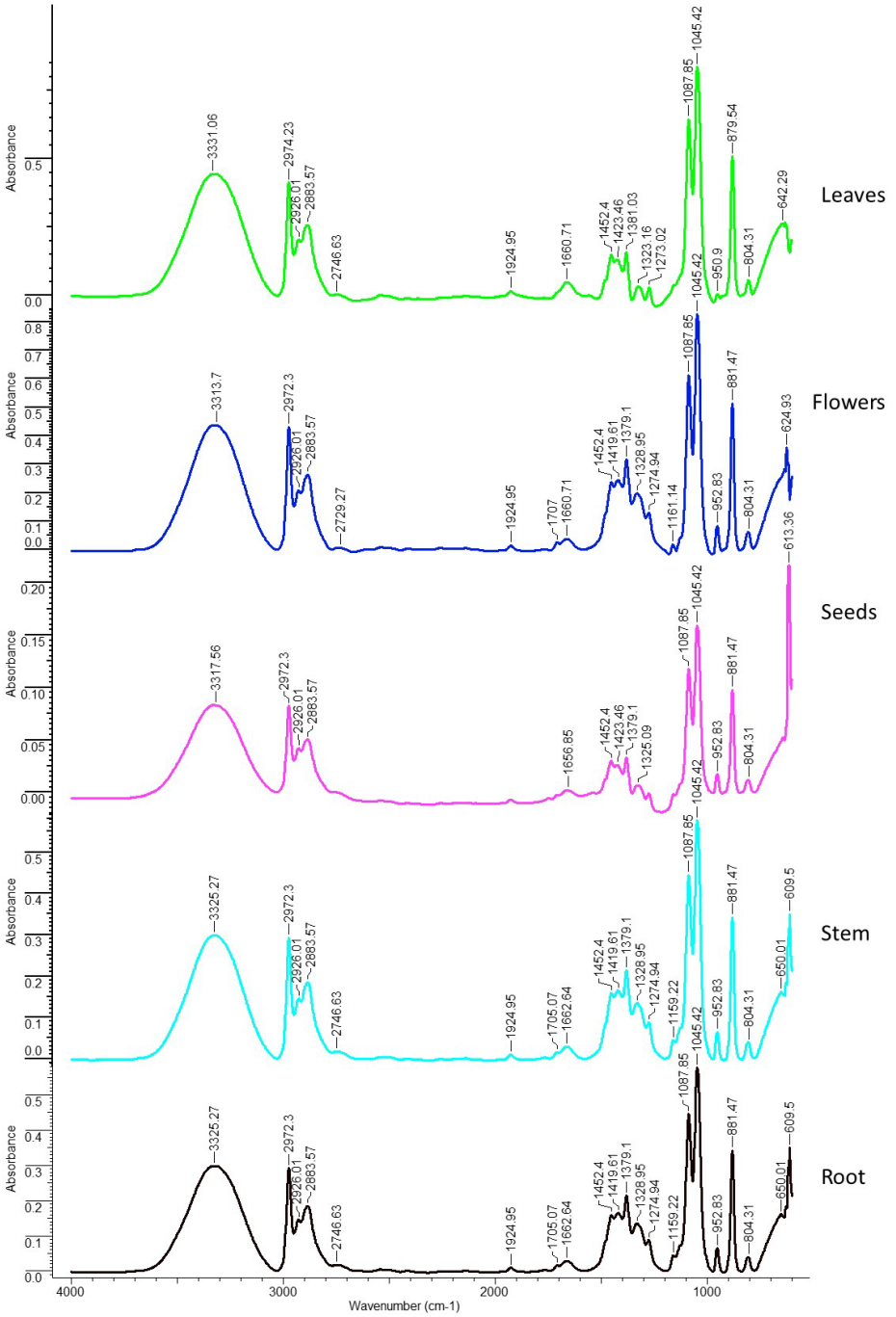


Figure 7: FTIR spectra of *Datura innoxia* leaf, flower, seed, stem and root extracts in EtOH

The absorbed peaks in the region of 1159 cm^{-1} and 1113 cm^{-1} indicate the C-N and C-C-C chemical bonds. It was also demonstrated that the absorption of the peaks at 1159 cm^{-1} and 1113 cm^{-1} is probably attributed to the presence of the C-O bond, characteristic of the ester group.[49] In the extracts of *Datura innoxia* plant biomass, they were identified in all plant organs extracted in 1-BuOH (Fig. 8), while for EtOH, only in leaves, stems and roots. The peak at 1070 cm^{-1} is attributed to the C–O stretching vibrations and C–OH bending vibration (EtOH extracts).[55] The peaks at 1045 cm^{-1} (EtOH) and 1042 cm^{-1} (1-BuOH) can be attributed to the C-N bond, characteristic of amines.[49]The bending vibrations for C–H (900–650 cm^{-1}) are characteristic of aromatic ring substitution[49,57,58] and correspond to terpenes,[39,44] which are identified in all plant extracts (Fig. 7 and Fig. 8).

The peak absorbance at 810 cm^{-1} is associated with the phenolic monoterpenoid, carvacol, identified only in flowers and seeds extracted in 1-

BuOH (Fig. 8).[48] Another characteristic peak is the one at 739 cm^{-1}, which can be associated with the hexaterpene, characteristic of the chemical compound α-bisabolol, identified only in 1-BuOH extracts.[48] At the same time, it was also demonstrated that the absorption of the peak at 739 cm^{-1} shows the out-of-plane bonding of the CH group, resulting in carbonyl compounds.[49] The peaks between 652–685 cm^{-1} correspond to the absorption band for alkenes.[55]

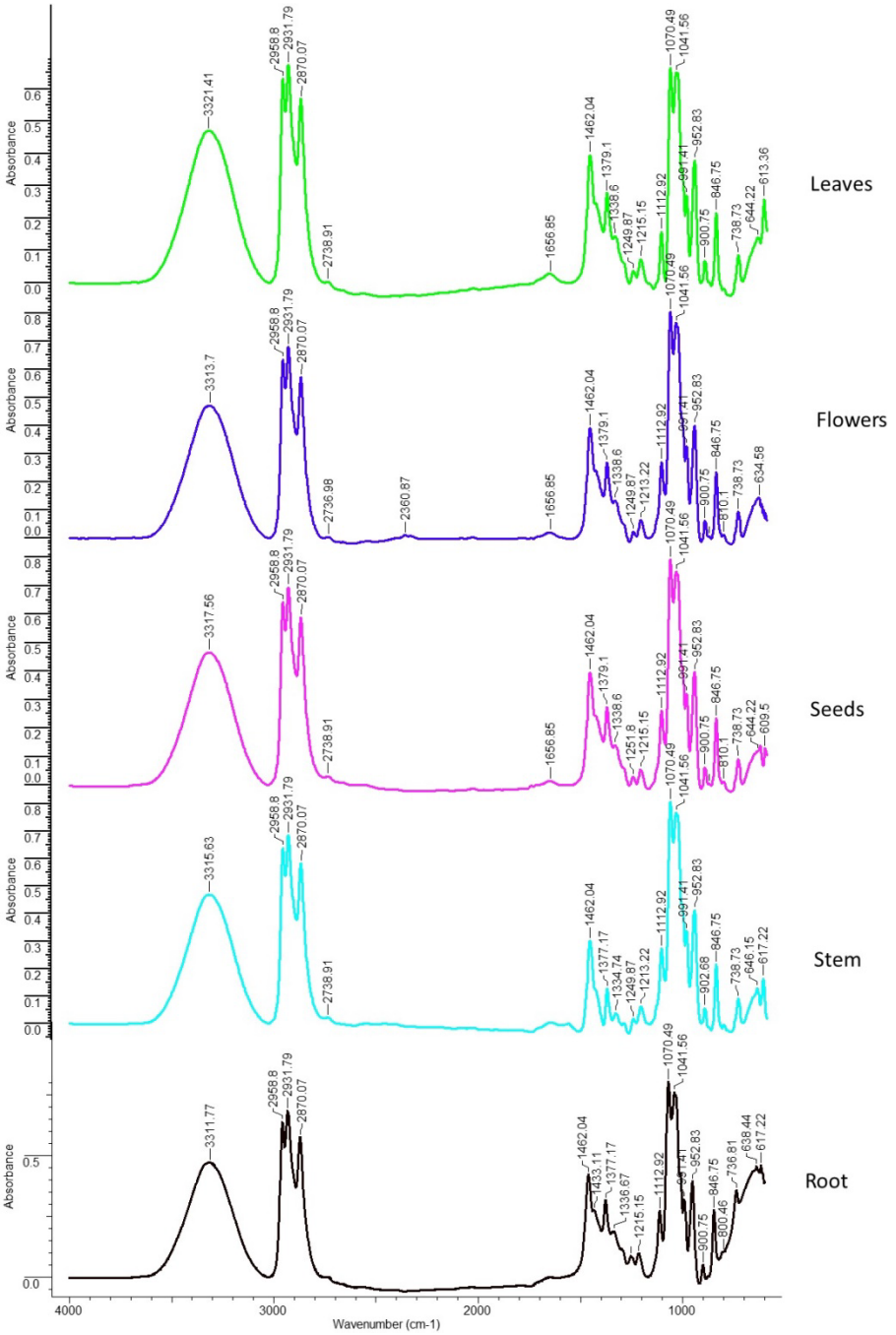


Figure 8: FTIR spectra of of *Datura innoxia* leaf, flower, seed, stem and root extracts in 1-BuOH

The FTIR spectra for the EtOH extracts (Fig. 7) and 1-BuOH extracts (Fig. 8) of the dried plant biomass of *Datura innoxia* show the complexity and diversity of primary and secondary metabolites in the plant. These spectra show almost the same absorption peaks, which can be confirmed that the same phytochemical compounds are present in the extracts obtained with both solvents. There are, however, minor deviations that can be observed in the spectra. The difference lies in the interaction of the phytochemical compounds with the solvent, which may explain the difference in the absorption

peaks of the phytochemical compounds present in the extracts.[49]

For UAE extraction, we also note that scopolamine was identified in all vegetative organs of the *Datura innoxia* plant using EtOH and 1-BuOH as solvents. The FTIR spectra presented a single fingerprint region for scopolamine, exhibiting the absorption peak at 881 cm^{-1} in EtOH (Fig. 7) and 847 cm^{-1} in 1-BuOH (Fig. 8). Thus, the characterization by FTIR analysis of the dry biomass extracts of *Datura innoxia* allows the identification of new compounds with applications in different fields.

Chromatographic analysis

GC-FID chromatograms were recorded for the extracts of the various plant tissues of *Datura innoxia* and are shown in Figure 9 for EtOH (Fig. 9(a)), and 1-BuOH (Fig. 9(b)), respectively. From these chromatograms, it can be seen that a good chromatographic separation regarding scopolamine was achieved, and the scopolamine chromatographic peak was identified at the retention time of approximately 12 min. It can be seen that scopolamine was identified in all plant organs, varying from one vegetative organ to another in the order: roots > flowers > leaves > seeds > stems. From a quantitative point of view, the results showed that the highest scopolamine content was determined for EtOH extracts of flowers (3142.15 ± 0.45 mg/kg dry weight), and for 1-BuOH extracts of leaves (7264.28 ± 0.69 mg/kg dry mass). The lowest amount was determined in the stem extracts in both EtOH and 1-BuOH. Figure 10 presents comparatively the amount of scopolamine determined in the *Datura innoxia* extracts (EtOH, 1-BuOH), using the non-conventional UAE extraction technique.

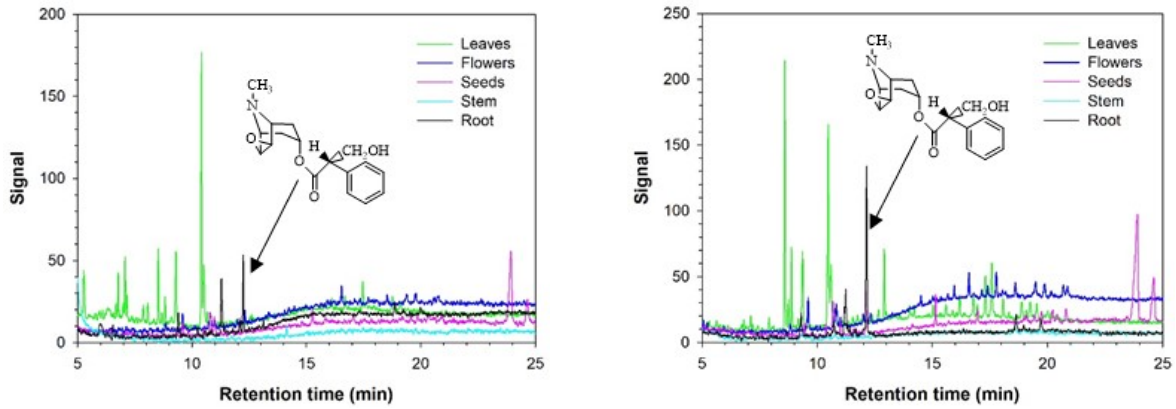


Figure 9: GC-FID chromatograms of *Datura innoxia* leaf, flower, seed, stem and root extracts in EtOH (a) and 1-BuOH (b) displaying the chromatographic peak of scopolamine

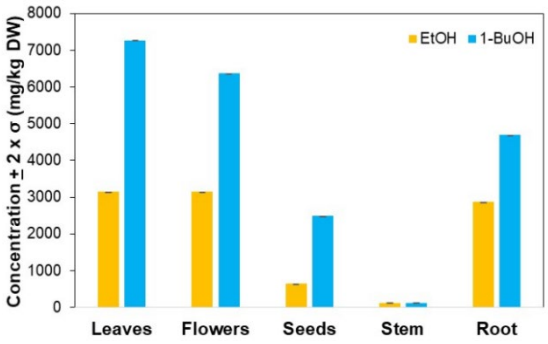


Figure 10: Scopolamine concentration expressed in mg/kg dry weight obtained in EtOH and 1-BuOH extracts of *Datura innoxia* plant biomass (error bars given as ± 2x standard deviation for 3 replicate measurements at the 95% confidence interval)

An overview of the data from the literature regarding *Datura* species[59-62] reveals that, until now, no study has been done (as far as we know) regarding the amount of scopolamine in the vegetative organs of the *Datura innoxia* plant adapted to the pedoclimatic conditions specific to the soil from Romania, using UAE as extraction technique. Taking this into account, scopolamine

concentration may depend on the geographical origin of the species.[63] The results obtained using UAE extraction led to the conclusion that it is an effective technique for scopolamine extraction from *Datura innoxia* plant biomass. When comparing the two solvents used, 1-BuOH has higher extraction efficiency than EtOH. Also, GC-FID led to the best scopolamine concentration results.

CONCLUSION

In this study, a non-conventional UAE extraction technique is applied for the extraction of bioactive compounds from *Datura innoxia* dry biomass, using two organic solvents (EtOH and 1-BuOH), and chromatographic, spectroscopic and spectrophotometric techniques were used for the characterization of the extracts.

The results show that scopolamine, the major tropane alkaloid, was identified in all vegetative organs, 1-BuOH being the solvent with higher extraction efficiency in this regard. For the characterization by GC-FID, the quantitative results showed higher concentration in the 1-BuOH leaf extract (7264.28 ± 0.69 mg/kg), compared with EtOH extracts. FL allowed the qualitative identification of compounds, specifically, of tocol derivatives found only in 1-BuOH flower extracts (λ_{em} = 308 nm), and chlorophyll (λ_{em} = 620 nm) found in EtOH and 1-BuOH extracts, and quantitatively, of scopolamine (370.09 ± 6.01 mg/kg) found in the leaf extracts. UV-vis allowed identifying secondary metabolites, such as alkaloids, phenols, carotenoids and chlorophyll. An important correlation was observed, especially in the spectral region between 223 nm and 661 nm, attributed to phenolic compounds and flavonoids (EtOH and 1-BuOH). As regards FTIR characterization, the results ensured rapid identification of the spectroscopic fingerprint area. Also, GC-FID led to the best scopolamine concentration results.

The data obtained during the experimental investigations led to important qualitative and quantitative information through the identification and characterization of some metabolites (alkaloids, terpenes, phenols, carbohydrates, tocopherols, tocotrienols *etc*.) from the plant biomass, which highlighted extremely interesting details related to the chemical composition. The results suggest the suitability of polar solvents for the extraction of secondary metabolites from *Datura innoxia* dry biomass. Particular attention was paid to the pharmacological potential of this plant, taking into account the fact that the presence of scopolamine – the alkaloid of interest, with implications in this sense – was highlighted in the chemical composition of the plant biomass.

Future studies will focus on the proposed biorefinery approach, which takes into account the full value of the biomass, due to the extraction of phytochemical compounds with high added value, and, simultaneously, by the transformation of the residual biomass from the extraction into fuels or platform chemicals.

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