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Recovery of Bioactive Compounds from the Biomass of Aromatic Plants After Distillation Using NADES: A Sustainable Alternative Extraction Method

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Abstract: The extraction processes for medicinal plants, particularly the distillation of aromatic plants, generate significant quantities of by-products, consisting of fibrous biomass and hydrosols. These by-products pose challenges for disposal and recovery. Consequently, it is imperative to make the entire, highly energy-intensive process more sustainable by valorizing all derivatives. This study aims to recover bioactive compounds, particularly polyphenols, from these biomasses. Polyphenols represent a large class of compounds known for their biological activities. *Artemisia dracunculus*, *Echinacea purpurea*, *Helichrysum italicum* (from the *Asteraceae* family), and *Lavandula angustifolia*, *Lavandula x intermedia*, *Melissa officinalis*, *Salvia officinalis*, *Salvia sclarea*, and *Salvia rosmarinus* (from the *Lamiaceae* family) were subjected to steam distillation. The essential oils obtained were characterized using gas chromatography, and the residual biomasses were processed with innovative extraction methods. The study investigated the use of natural deep eutectic solvents (NADES) for extracting polyphenols from the residual biomasses. Comparisons were made between the extracts obtained using NADES and those obtained using ethanol, a traditional solvent commonly employed for such purposes. The chemical characterization of the extracted compounds was performed using advanced analytical techniques, including HPLC-DAD and UHPLC-HRMS. The application of NADES demonstrated superior extraction efficiency for biomasses from both the *Asteraceae* and *Lamiaceae* families. Additionally, NADES exhibited several environmentally friendly characteristics, enhancing their sustainability profile. For these reasons, NADES present a viable alternative system for the recovery of bioactive compounds and could be used to formulate new products for the food, pharmaceutical, and cosmetic industries.

Keywords: NADES; *Lamiaceae*; *Asteraceae*; distillation; biomass; essential oils; HPLC-DAD; UHPLC-HRMS; GC-FID; GC-MS; polyphenols

1. Introduction

Aromatic plants have always been used in the therapeutic field due to the abundance of bioactive compounds contained in essential oil (EO). Currently, aromatic plants find employment in the pharmaceutic, cosmetic, food, and agriculture industries [1]. The main bioactive compounds in aromatic plants are terpenes and terpenoids, constituents of EOs, known for their antiseptic activity, medicinal properties, and fragrance. They are used as antimicrobial agents [2,3], analgesics [4], sedatives [5,6], anti-inflammatories [7], spasmolytics, local anesthetics, and as anti-cancer agents [8–10]. The EOs, as described by European Pharmacopeia, are volatile mixtures of odorous compounds, usually of complex composition, obtained from a botanically defined herbal drug by steam distillation, dry distillation, or a suitable mechanical process without heating, from roots, leaves, flowers, and fruit peels of aromatic plants. The extraction processes of the EOs from aromatic plants by means of industrial technique generate significant quantities of by-products, consisting of fibrous

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biomass and hydrosols [11]. Therefore, valorizing these wastes is extremely important to make the production of EOs more environmentally sustainable.

Currently, the exhausted biomasses are mainly employed for producing biofuel; however, these agri-food wastes can be exploited for more noble purposes being rich in bioactive compounds, such as polyphenols [12,13]. Polyphenols are a large class of chemical compounds that include phenolic acids, flavonoids, anthocyanins, proanthocyanins, and stilbenes with marked health benefits. Several studies have recently demonstrated the countless potentialities of polyphenols as therapeutic agents and food preservatives due to their anti-inflammatory, antimicrobial, antioxidant, and enzyme-inhibitory activities [14].

These compounds are usually extracted via conventional methods that employ inflammable, toxic, and contaminant organic solvents. In recent years, the research effort focused on the development of more sustainable strategies by using green technologies with higher process performances and solvents with a lower environmental impact. In this context, Natural Deep Eutectic Solvents (NADES) have been proposed as safe and environmentally friendly alternatives to classic solvents. The NADES rapidly showed potential in green chemistry due to their low cost, recyclability, biodegradability, biocompatibility, and non-toxicity. The NADES are eutectic mixtures composed of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) that create a dense bend network due to hydrogen bonding and van der Waals interactions [[15]. Therefore, NADES blends are capable of efficiently solubilizing lipophilic compounds and protecting thermolabile compounds. The efficiency and significance of NADES as solvents are attributed to the fact that these mixtures are inside cells. Indeed, when combined in appropriate ratios, mixtures of numerous primary metabolites can form natural deep eutectic solvents. The presence of NADESs inside cells is crucial because many macromolecules, such as DNA, proteins, and polysaccharides, which are poorly soluble in aqueous phase, can dissolve within these mixtures. Eutectic bends high solubilizing capacity is related to their molecular structure and wide polarity range. NADES can also play an important role in safeguarding organisms from harsh conditions, such as drought and cold [16]. In the last years, several studies have successfully demonstrated the potentiality of NADES in extracting polyphenols from aromatic plants. Specifically, polyphenol-rich extracts were obtained from different plants [17–20]. The employment of NADES in the recovery of bioactive compounds of aromatic plant by-products has not been considered so far. Furthermore, since NADES are totally biocompatible and non-toxic for humans, animals and the environment, it is important to further investigate their formulation in foods and feeds enriched with polyphenols and other bioactive compounds.

In this regard, the NADES were considered for the extraction of polyphenolic compounds from the biomasses of several *Lamiaceae* and *Asteraceae* plants widely distributed and cropped in the North of Italy for their characteristic aroma and therapeutic properties. Specifically, *Artemisia dracunculus* L. (ART), *Echinacea purpurea* (L.) Moench (ECHI), *Helichrysum italicum* (Roth) G. Don (HEL), *Lavandula angustifolia* Mill. (LAV), *Lavandula x intermedia* Emeric ex Loisel (LAI), *Melissa officinalis* L. (MEL), *Salvia officinalis* L. (SAO), *Salvia rosmarinus* Spenn. (ROS), and *Salvia sclarea* L. (SAS) were selected.

Different NADES formulations were prepared, and their extraction capability was compared to that of ethanol, a commonly extractive organic solvent to develop a sustainable extraction procedure for the recovery of bioactive compounds from oil-exhausted biomasses derived from the distillation of aromatic plants.

The chemical characterization of the EOs was made using gas-chromatography coupled with a mass spectrometer (GC-MS) and flame ionization (FID) detectors. The extraction capacity of NADES compared to traditional solvents was studied by Ultrahigh-Performance Liquid Chromatography–High-Resolution Mass Spectrometry (UHPLC-HRMS) to determine the polyphenolic composition, and High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) to quantify the characteristic active components of the plants belonging to the *Asteraceae* and *Lamiaceae* families.

2. Results

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2.1. Chemical Characterization of the EOs

The chemical characterization of the EOs obtained from the aerial parts of the aromatic plants was performed using GC–MS and GC-FID analysis. Table 1 summarizes the relative peak areas of each component, elution order, and comparison between experimental (exp) and literature (lit) LRI values.

The EOs belonging to the *Lamiaceae* family displayed a high content of oxygenated monoterpenes among which alcohols, aldehydes, ketones, and esters depending on the genus. *Lavandula x intermedia* and *Lavandula angustifolia* were mainly characterized by linalool (20.19% and 35.19% respectively) and its ester linalyl acetate (36.63% and 34.01% respectively). Also, both the EOs contained 1,8-cineole, cis-β-ocimene, trans-β-ocimene, fenchol, camphor, borneol, terpinen-4-ol, lavandulyl-acetate, β-caryophyllene and β-caryophyllene-oxide in characteristic and specific quantities that indicate a different biosynthetic pathway of the two species [21]. *Melissa officinalis* was characterized by high concentrations of aldehydes (citronellal 9.43%, geranial 12.74%, and neral 6.49) and caryophyllene derivatives as β-caryophyllene (17.08%) and caryophyllene-oxide (20.64%) [22].

The EO of *Salvia rosmarinus* showed a pinene chemotype due to the high concentration of the hydrocarbon monoterpene (α-pinene 38,79%) according to [23]. Significant percentages of 1,8-cineole (18.55%) and verbenone (6.04%) were detected in agreement with the literature [24].

Finally, the sage EOs displayed an extremely different chemical composition. Specifically, *Salvia officinalis* was represented by 38% of hydrocarbon monoterpenes, and the ketones camphor and α- and β-thujone which accounted for almost 38% of the total composition. Conversely, *Salvia sclarea* exhibited a chemical composition close to that of *Lavandula angustifolia*, being mainly characterized by linalool (15.87%) and its ester linalyl acetate (70.96%) [25].

Regarding the EOs extracted from the aerial parts of the *Asteraceae* plants as indicated by GC–MS characterization and GC-FID quantification, ART EO consisted of high amount of the phenylpropanoid estragol (65.15%) confirming the results of previous investigations [26]. Moreover, this oil was also characterized by monoterpene hydrocarbons, such as cis-ß-ocimene (11.50%), trans-ß-ocimene (15.70%). In ECHI EO, α-pinene, β-pinene, myrcene, and p-cymene were the most concentrated monoterpenes accounting for 15.76 % of the total composition. Conversely, germacrene D was the most abundant hydrocarbon sesquiterpene representing 66.43% of the total composition [27]. Concerning HEL EO, the most concentrated compounds were α-pinene, ar-curcumene, italidione II, limonene, β-caryophyllene, italicene, and α-selinene [28].

Table 1. Chemical composition of the essential oils obtained by steam distillation from *Lavandula x intermedia* (LAI), *Lavandula angustifolia* (LAV), *Melissa officinalis* (MEL), *Salvia rosmarinus* (ROS), *Salvia officinalis* (SAO), and *Salvia sclarea* (SAS), *Artemisia dracunculus* (ART), *Echinacea purpurea* (ECHI), and *Helichrysum italicum* (HEL).

Compound	LRI $_{exp}$	LRI $_{lit}$	LAI	LAV	MEL	ROS	SAO	SAS	ART	ECHI	HEL
α-thujene	928	928	-	-	-	0.25	0.19	-	-	-	-
α-pinene	935	936	0.28	0.60	0.18	38.79	3.84	-	1.53	5.56	24.53
Camphene	943	950	0.49	0.18	-	5.37	6.29	-	0.13	0.17	0.99
Sabinene	969	973	0.25	0.42	-	2.58	4.39	-	-	-	0.15
β-pinene	971	975	-	0.15	1.05	-	0.12	-	0.17	1.33	1.07
Octanone	988	985	0.61	0.43	-	0.62	-	-	-	-	0.17
Myrcene	989	992	0.97	1.21	0.54	2.55	2.00	1.40	0.15	7.98	-
α-phellandrene	1001	1005	-	-	-	0.30	-	-	-	0.76	-
δ-3-carene	1007	1010	0.12	0.48	-	-	-	-	-	-	-
α-terpinene	1017	1017	0.41	0.18	-	0.56	-	-	-	-	0.24
***p*-cymene**	1024	1026	0.67	0.29	0.16	0.40	0.40	0.23	-	0.89	0.29
Limonene	1029	1030	0.43	1.07	-	-	21.42	-	3.63	-	7.77
1,8-cineole	1031	1032	6.16	1.62	-	18.55	-	-	-	-	0.76
***cis*-β-ocimene**	1037	1039	0.49	3.04	-	-	-	0.37	11.50	-	-

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***trans*-β-ocimene**	1049	1049	0.26	4.04	-	-	-	0.63	15.70	-	0.32
γ-terpinene	1058	1061	0.11	0.13	-	1.12	-	-	-	-	0.71
Terpinolene	1085	1088	-	-	-	0.91	0.11	0.11	-	-	0.21
Linalool	1098	1102	20.19	35.19	0.61	1.82	0.15	15.87	-	-	0.87
α-thujone	1104	1101	-	-	-	-	10.70	-	-	-	-
6-camphenol	1105	1106	0.73	-	0.21	0.26	-	-	-	-	-
α-fenchol	1114	1116	2.22	1.04	0.25	-	-	-	-	-	-
β-thujone	1115	1112	-	-	-	-	5.70	-	-	-	-
***Trans*-rose oxide**	1126	1128	0.20	-	-	0.31	-	-	0.24	-	-
Camphor	1144	1145	3.52	0.35	-	3.83	22.41	-	-	-	-
Nerol oxide	1153	1148	-	-	-	-	-	-	-	-	0.72
Citronellal	1155	1157	-	-	9.43	0.17	-	-	-	-	-
Menthone	1157	1155	-	-	0.22	1.97	-	-	-	-	-
Borneol	1166	1168	1.80	1.32	-	3.79	1.62	-	-	-	-
Lavandulol	1169	1172	0.25	-	-	0.78	-	-	-	-	-
Terpinen-4-ol	1175	1181	1.01	5.59	-	0.78	0.43	-	-	-	0.31
***p*-cymen-8-ol**	1188	1185	0.24	-	0.76	-	0.19	-	-	-	-
α-terpineol	1189	1193	0.34	0.64	-	1.03	-	2.09	-	-	0.35
Myrtenal	1194	1195	0.31	0.15	-	0.29	-	-	-	-	-
Estragole	1200	1195				-			65.15		
Verbenone	1207	1204				6.04					
Nerol	1230	1232	-	-	0.18	-	-	0.19	-	-	1.04
Citronellol	1233	1233	-	-	0.18	-	-	0.15	-	-	-
Neral	1245	1245	0.15	-	6.49	-	-	-	-	-	-
Piperitone	1255	1252	0.13	-	0.50	1.23	-	-	-	-	-
Linalyl acetate	1264	1259	38.63	34.01	0.30	-	-	70.96	-	-	-
Geraniol	1267	1267	0.15	-	2.02	-	-	-	-	-	-
Geranial	1274	1269	-	-	12.74	-	-	-	-	-	-
Bornyl acetate	1288	1288	0.32	0.80	0.33	1.32	1.50	-	0.11	-	-
Lavandulyl acetate	1294	1290	3.13	-	-	-	-	-	-	-	-
δ-elemene	1338	1337	0.73	-	0.24	-	-	-	-	0.27	-
Neryl acetate	1367	1368	0.21	0.29	-	-	-	0.73	-	-	1.54
α-copaene	1377	1376	0.29	-	0.69	-	0.11	0.36	-	0.24	1.31
Geranyl acetate	1385	1386	-	-	3.50	-	-	1.43	-	-	-
β-bourbonene	1389	1384	-	-	0.41	-	0.11	0.20	-	-	-
β-cubebene	1392	unknown	0.44	0.67	0.39	-	-	-	-	0.94	-
β-elemene	1395	1390	0.20	-	-	-	-	0.14	-	-	-
Italicene	1404	1409	-	-	-	-	-	-	-	-	2.65
Cis-α-bergamotene	1416	1415	-	-	-	-	-	-	-	-	0.86
β-caryophyllene	1420	1420	1.97	3.30	17.08	2.46	4.62	0.99	0.23	2.50	3.49
α-humulene	1431	1433	-	0.34	1.93	-	-	-	-	0.65	-
Trans-α-bergamotene	1440	1436	0.12	0.26	-	-	1.80	-	-	-	-
Neryl propanoate	1455	1452	-	-	-	-	-	-	-	-	2.47
β-farnesene	1456	1457	1.20	-	0.15	-	-	-	-	1.13	0.31
alloaromadendrene	1464	1466	-	-	-	0.34	0.23	-	-	-	-
γ -curcumene	1478	1480	-	-	-	-	-	-	-	-	2.01
ar-curcumene	1483	1484	0.37	0.43	-	-	0.15	-	0.38	-	9.85
β-selinene	1485	1489	-	-	-	-	-	-	0.25	-	2.41
Germacrene D	1488	1481	-	-	1.10	-	0.12	2.83	-	66.43	-
Italidione II	1491	1493	-	-	-	-	-	-	-	-	9.55
α-selinene	1499	1496	-	-	-	-	0.75	0.56	-	1.86	6.38
β-bisabolene	1510	1511	-	-	-	-	-	-	-	-	0.26

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β-curcumene	1513	1513	-	-	-	-	-	-	-	-	0.37
γ-cadinene	1522	1524	0.12	-	0.41	-	-	-	-	-	0.14
δ-cadinene	1526	1523	0.69	0.24	0.19	-	0.28	-	-	1.31	0.11
Nerolidol	1559	1563	-	-	0.97	-	-	-	-	-	-
Spathulenol	1580	1577	-	-	-	-	0.76	-	-	0.57	-
Italidione III	1583	1583	-	-	-	-	-	-	-	-	0.96
Caryophyllene oxide	1590	1589	2.35	0.23	20.64	0.14	0.96	0.13	-	1.65	-
TOTAL			93.64	98.71	94.04	99.53	92.00	99.37	99.54	96.82	87.01

Experimental retention indices and literature retention indices (HP-5 column) according to NIST [29].

2.2. Optimization of NADES Extraction

The oil-exhausted aerial parts biomasses were extracted via ultrasonication using EtOH 70%, as conventional solvent, and the NADES formulations. Ultrasound-assisted extraction is considered an environmentally friendly methodology due to the shorter processing time and higher extraction yield compared to dynamic maceration, one of the most employed extraction techniques [30]. The higher recovery of polyphenol is ascribable to the cavitation phenomenon, which induces the formation, growth, and collapse of cavitation bubbles. The cavitation bubbles promote the disruption of the cell walls of the plant material and increase the contact area with the solvent, resulting in a fast release of bioactive compounds. NADES formulations were prepared selecting ChCl as the HBA. ChCl is one of the most popular natural HBA due to its affordability, biodegradability, safety, and health-beneficial effects. Also, the European directive 70/524/EEC8 authorized the employment of ChCl in feeds as an additive without time limitation [31]. ChCl was proposed in combination with several organic acids, among which lactic and citric acids, to extract polyphenols from foods and aromatic plants [32–36]. Lactic and citric acids were selected among the other organic acids for their low toxicity and low costs. Moreover, NADES formulations composed of these organic acids have been reported to efficiently extract polyphenols due to the polarity of the resulting eutectic solvent [37,38] . Since the ultrasonic variables have a strong impact on the extraction performances of NADES, temperature and time of extraction were kept fixed to evaluate only the contribution of the chemical composition of eutectic solvents [30]. The molarity of ChCl and the amount of water were kept fixed and their ratio with lactic and citric acids was varied to modulate to some extent the NADES properties, such as polarity and extraction affinity.

The extraction performance of NADES formulations and EtOH was investigated by HPLC-DAD determining the concentration of the most representative phenolic acid. To evaluate which NADES mixture has the highest extraction capacity, the reference samples used were ECHI and MEL for the *Asteraceae* and *Lamiaceae* families respectively. Specifically, chicoric acid and rosmarinic acid contents within the various were considered, accordingly with the literature [39] (Figure 1).

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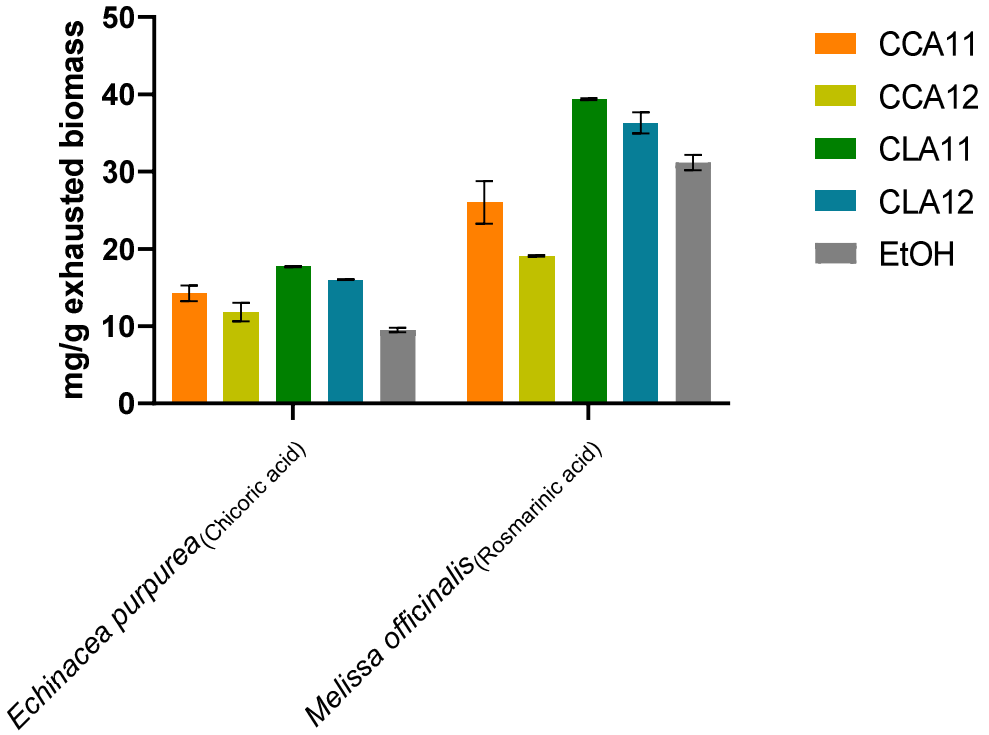

Figure 1. Amount of chicoric and rosmarinic acids extracted with ethanol and NADES formulations in *Echinacea purpurea* and *Melissa officinalis* biomasses.

Overall, the weakest NADES was CCA12 followed by CCA11. In the case of MEL, these eutectic formulations were less efficacious than EtOH in extracting rosmarinic acid. In the case of ECHI, EtOH achieved the lowest recovery. The extraction of chicoric acid with ChCl and lactic acid at the molar ratio 1:1 was significantly higher than that obtained with EtOH or the other NADES formulation ($P < .05$). The same results were obtained for the rosmarinic acid in MEL ($P < .05$). The variation of the ratio of lactic acid with the HBA did not significantly affect the extraction of rosmarinic acid in MEL; conversely, in the case of ECHI a significant difference was highlighted ($P < .05$). The greater extractive capacity of lactic acid compared to citric acid has already been reported in the literature on different natural matrices [40–42]. These differences might be related to various factors affecting the extraction capacity, such as viscosity, polarity, pH, and the number of hydrogen bond acceptor and donator groups. Lactic acid and citric acid differ in the number of carboxylic groups and the physical state. Citric acid is a powder. Therefore, the presence of high concentrations of citric acid increases the viscosity of the resulting NADES. Moreover, viscosity is strictly correlated to the number of carboxylic acid functional groups. Thus, the higher viscosity of citric-based NADES than lactic-based NADES could have impaired the extractive performance. The high viscosity of NADES has been reported to hinder the extractive efficiency [43] and decrease the mass transfer and diffusivity of compounds. The number of functional groups also affects the pH of the solvent in addition to the viscosity. Overall, acidic conditions are preferable for the extraction of polyphenols, as these compounds remain in their non-dissociated form at low pH. However, highly acidic NADES with a pH close to 0, such as ChCl/citric acid, may hinder the interaction of phenols. These factors could also explain why, in our study, the NADES with a 1:1 molar ratio of ChCl to lactic acid exhibited slightly greater extractive strength [44].

2.3. Analysis of Optimized NADES and EtOH Extracts

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All the biomasses were extracted using the NADES formulation containing lactic acid at a 1:1 molar ratio with ChCl, as it provided the highest recovery of the most characteristic polyphenols. The EtOH and NADES extracts were qualitatively analyzed by UHPLC-HRMS to identify all metabolites.

The polyphenolic profile of the extracts was characterized by the presence of compounds from the phenolic acid and flavonoid classes. Overall, the NADES extracts contained a greater number of metabolites than the EtOH extracts, due to the higher extractive power of the eutectic solvent, as shown in Table 2. Additionally, the *Lamiaceae* biomasses exhibited a greater diversity of metabolites compared to the *Asteraceae* biomasses.

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Table 2. Chemical composition of waste distillation biomasses extracted by EtOH 70% or CLA11 by UHPLC-HRMS.

No	Rt (min)	Molecule	(M-H) (m/z)	Error (ppm)	Fragments (m/z)	Formula	Molecular weight (g/mol)	Extract		Reference
									EtOH	NADES	
1	2.52	Danshensu	197.0449	0.70	179.0342, 135.0441, 123.0440, 72.9918	C9H10O5	198.052824	LAI	+	+	[44]
								LAV	+	+	
								MEL	+	+	
								ROS	+	+	
								SAO	+	+	
								SAS	+	+	
								ART	-	-	
								ECHI	-	-	
								HEL	-	-	
2	2.6	Protocatechuic acid hexose	315.0723	-2.19	153.0184, 152.0106, 109.0282, 108.0205	C13H16O9	316.079435	LAI	+	+	[45]
								LAV	+	+	
								MEL	+	+	
								ROS	+	+	
								SAO	+	+	
								SAS	+	+	
								ART	+	+	
								ECHI	+	+	
								HEL	-	-	
3	3.16	Caftaric acid	311.0408	-1.58	179.0342, 149.0082, 135.0441	C13H12O9	312.048135	LAI	+	+	[46]
								LAV	-	-	
								MEL	-	-	
								ROS	-	-	
								SAO	-	-	
								SAS	-	-	
								ART	-	-	
								ECHI	+	+	
								HEL	-	-	
4	3.79	Caffeoylquinic acid isomer I	353.0876	-0.96	191.0554, 179.0342, 135.0441	C16H18O9	354.095085	LAI	-	-	[45]
								LAV	-	-	
								MEL	-	-	
								ROS	+	+	
								SAO	+	+	
								SAS	-	-	
								ART	+	+	
								ECHI	-	-	
								HEL	+	+	

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5	4.34	Caffeoyl hexose isomer I	341.0878	-1.58	179.0342, 161.0235, 135.0442	C15H18O9	342.095085	LAI LAV MEL ROS SAO SAS ART ECHI HEL	- - + + + - - - +	+ - + + + - + + -	[47]
6	4.79	Coumaroyl quinic acid	337.0931396	-2.36	191.0555, 163.0392, 119.0490, 93.0333	C16H18O8	338.100170	LAI LAV MEL ROS SAO SAS ART ECHI HEL	- - - - - - + - +	- - - - - - + - +	[48]
7	5.01	Coumaroyl hexose	325.0929	-1.71	183.0114, 163.0392, 119.0492, 93.0333	C15H18O8	326.100170	LAI LAV MEL ROS SAO SAS ART ECHI HEL	+ + + + - - + - +	+ + + + + - + - +	[49]
8	5.35	Caffeoyl hexose isomer II	341.0877	-1.29	179.0342, 161.0235, 135.0441	C15H18O9	342.095085	LAI LAV MEL ROS SAO SAS ART ECHI HEL	- - + + + - - - -	+ - + + + - + + -	[47]
9	5.66	Caffeoylquinic acid isomer II	353.0876	-0.96	191.0554, 179.0342, 135.0441	C16H18O9	354.095085	LAI LAV MEL ROS SAO SAS ART ECHI	- - - + + - + -	- - - + + - + -	[45]

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								HEL	+	+	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	-	-	
10	6.42	Feruloylquinic acid	367.1031	-0.52	193.0499, 149.0598, 134.0363	C17H20O9	368.110735	SAO	-	-	[50]
								SAS	-	-	
								ART	+	+	
								ECHI	-	-	
								HEL	-	-	
								LAI	+	+	
								LAV	+	+	
								MEL	-	-	
								ROS	+	+	
11	6.48	Feruloyl hexose	355.1035	-1.66	193.0499, 149.0598, 134.0362, 119.0339	C16H20O9	356.110735	SAO	+	+	[49]
								SAS	-	-	
								ART	+	+	
								ECHI	-	-	
								HEL	-	-	
								LAI	-	+	
								LAV	-	+	
								MEL	-	-	
								ROS	-	-	
12	8.92	Luteolin /kaempferol diglucuronide	637.1053	-1.89	461.0721, 285.0405, 255.0298, 227.0349	0	638.111920	SAO	+	+	[49]
								SAS	-	-	
								ART	-	-	
								ECHI	-	-	
								HEL	-	-	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	-	-	
13	9.32	Myricetin hexose	479.0837	-2.36	317.0305, 271.0250	C21H20O13	480.090395	SAO	-	-	[50]
								SAS	-	-	
								ART	-	-	
								ECHI	-	-	
								HEL	+	+	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
14	9.63	Quercetin glucuronide	477.0680	2.63	301.0359, 300.0286, 271.02567, 255.0287	C21H18O13	478.074745	ROS	-	-	[51]
								SAO	+	+	
								SAS	-	-	

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15	9.68	Quercetin hexose	463.0888	-2.47	301.0359, 300.02719, 271.02567, 255.02921	C21H20O12	464.095480	ART ECHI HEL LAI LAV MEL ROS SAO SAS	- - - - - - + -	- - - - - - + -	[45]
16	9.78	Yunnaneic acid D	539.1196	-1.20	359.0777, 297.0771, 197.0452, 179.0342, 161.0236, 135.0441	C27H24O12	540.126780	ART ECHI HEL LAI LAV MEL ROS SAO SAS	- - + + - + + -	- - + + + + + -	[52]
17	9.86	Chicoric acid	473.0692	5.93	311.0411, 293.0308, 179.0343, 149.0082	C22H18O12	474.079830	ART ECHI HEL LAI LAV MEL ROS SAO SAS	- - - - - + - -	- - - - - + - -	[53]
18	10.02	Yunnaneic acid F	597.1254	-1.62	311.0930, 197.0449, 179.0342, 135.0441	C29H26O14	598.132260	ART ECHI HEL LAI LAV MEL ROS SAO SAS	- + - - - - + + -	- + - + - - + + -	[54]
19	10.51	Rutin	609.1468	-2.03	300.0278, 271.0251, 255.0301	C27H30O16	610.153390	ART ECHI HEL LAI LAV MEL ROS SAO	- - - - - - + -	- - - + - - + -	[55]

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20	10.74	Luteolin /kaempferol hexose	447.0939	-2.60	285.0405, 255.0298, 227.0349	C21H20O15	448.100564	SAS	-	+	[56]
								ART	+	+	
								ECHI	+	+	
								HEL	-	-	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	-	-	
								SAO	+	+	
								SAS	+	+	
21	10.90	Luteolin /kaempferol glucuronide	461.0731	-2.37	285.0405, 255.0298, 227.0349	C21H18O12	462.079830	ART	-	-	[49]
								ECHI	-	-	
								HEL	+	+	
								LAI	+	+	
								LAV	+	+	
								MEL	-	+	
								ROS	+	+	
								SAO	+	+	
								SAS	+	+	
22	11.06	Rosmarinic acid hexose	521.1304	-1.69	359.0766, 197.0449, 179.0342, 161.0236, 135.0440	0	521.129520	ART	-	-	[55,57,58]
								ECHI	-	-	
								HEL	-	-	
								LAI	-	-	
								LAV	-	-	
								MEL	+	+	
								ROS	+	+	
								SAO	+	+	
								SAS	-	-	
23	11.81	Luteolin /kaempferol rutinose	593.15184	1.09	285.04041, 255.02995, 227.03470,	C27H30O15	594.15912	ART	-	-	[59]
								ECHI	-	-	
								HEL	-	-	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	-	+	
								SAO	+	+	
								SAS	-	-	
24	11.92	Rosmarinic acid	359.0776	-2.52	197.0449, 179.0344, 161.0235, 135.0441	C18H16O8	360.084520	ART	+	+	[60]
								ECHI	-	-	
								HEL	+	+	
								LAI	+	+	
								LAV	+	+	
								MEL	+	+	
								ROS	+	+	

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25	12.09	Apigenin hexose	431.0988	-2.37	269.0454, 117.0332	C21H20O10	432.105649	SAO	+	+	[56]
								SAS	+	+	
								ART	-	+	
								ECHI	-	-	
								HEL	-	-	
								LAI	+	+	
								LAV	+	+	
								MEL	-	+	
								ROS	-	-	
								SAO	-	-	
								SAS	+	+	
								ART	-	-	
								ECHI	-	-	
								HEL	-	-	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	-	-	
26	12.30	Apigenin glucuronide	445.0782	-2.49	269.0457, 117.0334	C21H18O11	446.084915	SAO	-	+	[54]
								SAS	+	+	
								ART	-	-	
								ECHI	-	-	
								HEL	-	-	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	-	-	
27	12.43	Dicaffeoyl quinic acid	515.14105	0.73	353.08780, 191.05553, 179.03413, 135.04416	C25H24O12	516.147900	SAO	-	-	[59]
								SAS	-	-	
								ART	+	+	
								ECHI	-	-	
								HEL	+	+	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	-	-	
28	12.72	Coumaroyl-caffeoylquinic acid	499.1250	-1.92	353.0886, 337.0932, 191.0555, 173.0447	C25H24O11	500.131865	SAO	-	-	[61]
								SAS	-	-	
								ART	+	+	
								ECHI	-	-	
								HEL	-	-	
								LAI	-	-	
29	12.86	Methylluteolin-O-glucuronide	475.0888	-2.41	299.0561, 284.0327	C22H20O12	476.095480	LAV	-	-	[62,63]
								MEL	-	-	

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		(Kaempferide glucuronide)						ROS SAO SAS ART ECHI HEL LAI LAV MEL	+ + + - - - + - +	+ + + - - - + - +	
30	12.89	Salvianolic acid K	555.1122	3.01	359.0778, 179.0341, 161.0235, 135.0441	C27H24O13	556.121695	ROS SAO SAS ART ECHI HEL LAI LAV MEL	- + + - - - - - -	+ + + - - - - - -	[62]
31	13.28	Caffeoyl-feruloylquinic acid	529.1356	-1.88	367.1036, 179.0340, 161.0236, 135.0442	C26H26O12	530.142430	ROS SAO SAS ART ECHI HEL LAI LAV MEL	- - - + - - - - -	- - - + - - - - +	[61]
32	13.47	Salvianolic acid H (lithospermic acid)	537.1042	-1.67	359.0777. 295.0613. 179.0334. 161.0234. 135.0441	C27H22O12	538.111130	ROS SAO SAS ART ECHI HEL LAI LAV MEL	- + + - - - + - -	+ + + - - - + - +	[64]
33	15.30	Salvianolic acid A	493.1140	-1.06	295.0613, 197.0451, 179.0343, 135.0442	C26H22O10	494.121300	ROS SAO SAS	+ + -	+ + +	[44]

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34	15.33	Sagecoumarin isomer I	535.0882	-1.02	359.0775, 197.0443, 179.0341, 177.0186, 161.0234, 135.0443	C27H20O12	536.095480	ART ECHI HEL LAI LAV MEL ROS SAO SAS	- - - - - + - + -	- - - - - + - + +	[65]
35	16.03	Sagecoumarin isomer II	535.0882	-1.02	359.0775, 197.0443, 179.0341, 177.0186, 161.0234, 135.0443	C27H20O12	536.095480	ART ECHI HEL LAI LAV MEL ROS SAO SAS	- - - - - + - + -	- - - - - + - + +	[62]
36	16.21	Salvianolic acid C	491.0989	-2.14	311.0566, 265.05, 179.0360, 135.0442	C26 H20 O10	492.105649	ART ECHI HEL LAI LAV MEL ROS SAO SAS	- - - - - + + - -	- - - + - + + - +	[44]
37	12.13	Salvianolic acid B	717.1473	-2.42	519.0939, 339.051 161.023, 135.0444	C36H30O16	718.153390	ART ECHI HEL LAI LAV MEL ROS SAO SAS	- - - + - + - + +	- - - + - + - + +	[52]
38	12.31	Sagerinic acid	719.1624	1.64	359.0776, 197.0449, 179.0342, 161.0235	C36H32O16	720.169040	ART ECHI HEL LAI LAV MEL ROS SAO	- - - + - + + +	- - - + - + + +	[62]

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39	15.83	Micropyrone	251.1289	2.39	207.1385, 151.1118, 113.0960	C14H20O4	252.136160	SAS	+	+	[66]
								ART	-	-	
								ECHI	-	-	
								HEL	-	-	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	-	-	
								SAO	-	-	
								SAS	-	-	
								ART	-	-	
								ECHI	-	-	
								HEL	+	+	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	+	+	
40	22.70	Rosmadial (safficinolide)	343.1552	-1.75	299.16509	C20 H24 O5	344.162375	SAO	+	+	[67]
								SAS	-	-	
								ART	-	-	
								ECHI	-	-	
								HEL	-	-	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	+	+	
41	22.88	Carnosol	329.1758	-1.60	285.1862	C20H26O4	330.183110	SAO	+	+	[60,68]
								SAS	-	-	
								ART	-	+	
								ECHI	-	-	
								HEL	-	-	
								LAI	-	-	
								LAV	-	-	
								MEL	-	-	
								ROS	-	-	
42	23.52	Arzanol	401.1609	-2.17	247.0976, 235.0974, 191.1071, 166.0263, 153.0548, 109.0647	C22H26O7	402.167855	SAO	-	-	[66]
								SAS	-	-	
								ART	-	-	
								ECHI	-	-	
								HEL	+	+	

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The phenolic acids were represented by hydroxycinnamic acid derivatives except for the protocatechuic acid hexose (**2**), a dihydroxybenzoic acid. The hydroxycinnamic class was constituted by esters of caffeic, coumaric, and ferulic acids or their derivatives. Compounds **1**, **2**, **3**, **4**, **5**, **8**, **9**, **16**, **17**, **18**, **22**, **24**, **26**, **27**, **28**, **31**, **32**, **33**, **34**, **35**, and **36** were identified as caffeic acid derivatives due to the presence of the characteristic fragment ions at *m/z* 179.034, 161.023, and 135.044. Specifically, the parent ion **1** at *m/z* 197.045 was assigned to the hydration product of caffeic acid, danshensu (3,4-dihydroxyphenyl lactic acid), due to the loss of the hydroxylic group (18 Da). Metabolites **4** (and **9**) and **5** (and **8**) were recognized as caffeoylquinic acid and caffeoyl hexose respectively. The fragments ions of the caffeic acid were generated by the neutral loss of the quinic acid (– 191 Da) and the hexose (– 162 Da) moieties. Similarly, compound **3** was identified as caftaric acid (caffeoyltartaric acid) because of the neutral loss of 132 Da ascribable to the tartaric acid moiety. All the other caffeic acid derivatives were the product of the condensation of caffeic acid with one or more other phenolic acids. The most abundant derivative in the extracts of *Lamiaceae* biomasses was the rosmarinic acid. The peaks **24** and **22** were assigned to rosmarinic acid and its hexoside. Rosmarinic acid was characterized by the precursor ion at *m/z* 359.076 that yielded the daughter ions at *m/z* 197.044 and 179.034 that correspond to the deprotonated danshensu and caffeic acid respectively. The hexoside at *m/z* 521.034 underwent the loss of hexose moiety (– 162 Da) and generated the characteristic fragments of the rosmarinic acid. Compounds **30**, **32**, **33**, **36**, and **37** were recognized as salvianolic acids, other characteristic metabolites of *Lamiaceae* generated from the condensation of hydroxycinnamic acids. Metabolite **37** was found the product of the condensation of two rosmarinic acids. The differences within these metabolites are the ratios between danshensu and caffeic acid [62]. Caffeic acid was also found condensed with a coumarin in peaks **34** and **35**.

The parent ions **16** and **18** at *m/z* 539.119 and 597.125 were assigned to yunnaneic acid D and F respectively. From the precursor ion **16**, a neutral loss of caffeic acid yielded the fragment at *m/z* 359.077 (rosmarinic acid). Yunnaneic acid was putatively identified for the presence of the characteristic fragmentation ions at *m/z* 311.093, 197.045, 179.034, and 135.044 [44]. Chicoric acid (dicaffeoyltartaric acid) was assigned to peak **17** at *m/z* 473.069 that yielded the fragment peak at *m/z* 311.041 (– 162 Da, caffeoyl moiety) corresponding to the deprotonated caftaric acid. Similarly, metabolite **27** was recognized as dicaffeoylquinic acid due to the neutral loss of a caffeoyl moiety which yielded the fragment at *m/z* 353.087, the deprotonated form of caffeoylquinic acid. Compound **28** was recognized as coumaroyl-caffeoylquinic acid because of the neutral loss of the coumaroyl moiety (– 146 Da) that produced the deprotonated caffeoylquinic acid at *m/z* 353.088. Similarly, metabolite **31** at *m/z* 529.135 was identified as caffeoyl-feruloylquinic acid due to the neutral loss of a caffeoyl moiety (– 162 Da). The generated fragment at *m/z* 367.103 was the deprotonated form of feruloylquinic acid. This latter ion was found as precursor ion in peak **10**. The compound was tentatively recognized due to the typical daughter peaks of ferulic acid at *m/z* 193.049, 149.059, and 134.036. Also, compound **11** was identified as ferulic acid derivative. The parent ion at *m/z* 355.103 yielded the characteristic fragments of ferulic acid because of the loss of the hexoside (– 162 Da). Similarly, compound **7** was tentatively identified as coumaroyl hexose due to the presence of the characteristic fragment ions of coumaric acid at *m/z* 163.039, 119. 049, and 93.033. Finally, the parent ion **6** at *m/z* 337.093 was assigned to coumaroylquinic acid because of the additional presence of the peak at *m/z* 191.055 related to the deprotonated form of quinic acid (loss of coumaroyl moiety, – 146 Da).

Regarding the flavonoid class, derivatives of quercetin, kaempferol/luteolin, myricetin, and apigenin were present. Metabolites **14** and **15** were recognized as quercetin glucuronide and quercetin hexose because of the presence of quercetin fragment ions at *m/z* 301.035, 300.027, 271.024, and 255.029 generated from the loss of glucuronic acid and hexose moiety (– 176 Da and – 162 Da respectively). The peak at *m/z* 609.146 (**19**) was assigned to rutin (quercetin rutinoside) due to the neutral loss of rutinoside moiety (– 308 Da).

Kaempferol/luteolin derivatives (**12**, **20**, **21**, and **23**) were identified for the characteristic fragment peaks of the aglycone at *m/z* 285.040, 255.029, 227.034. The parent ions at *m/z* 637.105 (**12**),

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447.093 (**20**), 461.073 (**21**), and 593.151 (**23**) were recognized for the neutral loss of two consecutive glucuronic acid moieties (– 176 Da), hexose (– 162 Da), one glucuronic moiety (– 176 Da), and rutinoside moiety (– 308 Da), respectively.

Myricetin hexose was assigned to the parent ion **13** at *m*/*z* 479.083 because of the fragment peaks at *m*/*z* 317.030 (loss of the hexose, – 162 Da) and 271.025.

Apigenin hexose and glucuronide (**25** and **26**) were identified for the aglycone characteristic daughter ions at *m*/*z* 269.045, 117.033 generated from the loss of and hexose (– 162 Da) and a glucuronic moiety (– 176 Da) respectively.

Finally, metabolites **40** and **41** were found in ROS and SAO biomasses and were identified as phenolic diterpenes, with a fragmentation pattern consistent with the literature [62]. Conversely, micropyrone (**39**) and arzanol (**42**) was tentatively identified in according to Kramberger et al. only in HEL biomass as they are among the most characteristic metabolites of this plant [66].

From the qualitative analysis, rosmarinic acid was the most abundant phenolic acid in all *Lamiaceae* biomasses. Besides, polyphenolic profiles of DRA and HEL [69–72] were mostly represented by dicaffeoylquinic acid as reported by other authors also in different species of the genus [73,74].

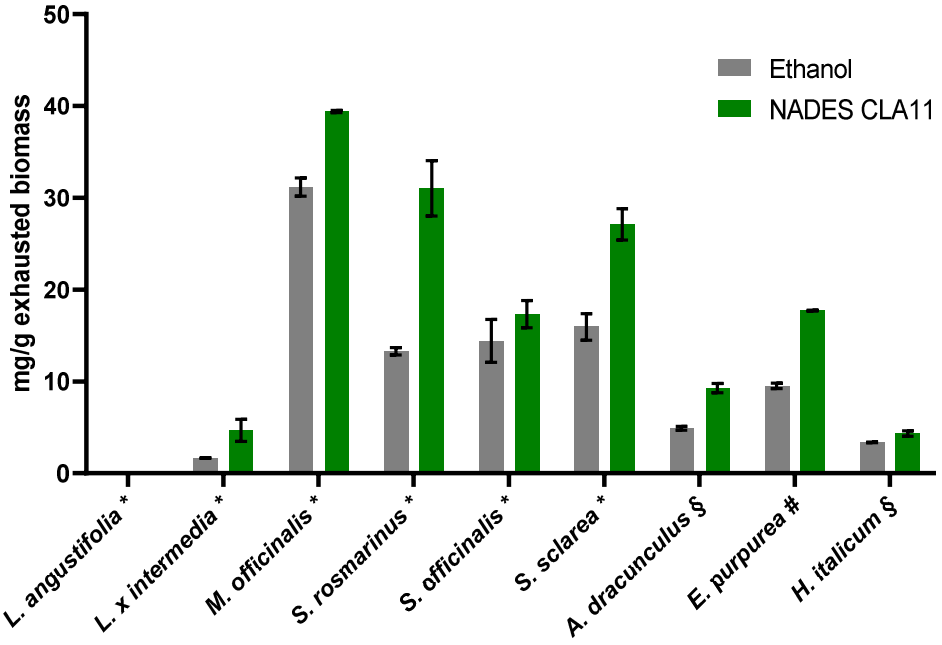

Figure 2. Amount of the most characteristic polyphenols extracted with EtOH and NADES CLA11 in all biomasses. * rosmarinic acid; § dicaffeoylquinicacid; # chicoric acid.

The concentration of the target polyphenols was higher in all NADES extracts compared to the EtOH extracts, confirming the preliminary results on ECHI and MEL during the screening of the NADES formulations. In the case of LAV biomass, rosmarinic acid was not detected in both extracts, confirming the TPC results. The detection of some polyphenols in this biomass via UHPLC-HRMS is ascribable to the high sensitivity of the analytical method. The differences in the extractive performances of EtOH and NADES were not pronounced across all biomasses. However, the concentration of phenols in NADES extracts of LAI, ROS, SAS, DRA, and ECHI was significantly higher ($P < .05$) and nearly twice that of the EtOH extracts. In contrast, for SAO and HEL, the differences in polyphenol abundance were not significant, suggesting that NADES may not efficiently penetrate the plant material or solubilize the metabolites of interest.

In general, the greater recovery suggested that the polarity of NADES closely aligns with that of the phenolic compounds in the plant biomasses, influencing their capability to dissolve the metabolites. The enhanced extraction capacity of NADESs is attributed to the high number of

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hydrogen bonds between the polyphenols and the components of the mixtures. Therefore, the high solubility of these bioactive compounds within NADESs is primarily due to the stabilization resulting from the intermolecular interactions they form with the NADES bends [16,75].

3. Materials and Methods

3.1. Chemicals and Sample Materials

Chicoric acid and C_8–C_{40} n-alkanes mixture were provided from Sigma-aldrich (Milan, Italy). Lactic and citric acid were purchased from Carlo Erba (Milan, Italy). Choline chloride (ChCl) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Cynarine was obtained from LGC (North York, Canada), while caftaric acid from Dr EHRENSTORFER (Augusta, Germany). Acetonitrile (ACN), acetic acid (HAc), formic acid, n-hexane (Hex), and ethanol (EtOH) were of LC–MS purity grade (Sigma-Aldrich, Milan, Italy).

The aerial parts of *Artemisia dracunculus* (ART), *Salvia rosmarinus* (ROS), and *Lavandula x intermedia* (LAI) were provided by Giardino delle Erbe "Rinaldi Ceroni", Casola Valsenio (Ravenna, Italy) 6JJF+8H, *Echinacea purpurea* (ECHI), *Helichrysum italicum* (ELI), *Lavandula angustifolia* (LAV), *Melissa officinalis* (MEL), *Salvia officinalis* (SAO), and *Salvia sclarea* (SAS) were provided by "La Bendessa" farm, Roncoscaglia, Sestola (Modena, Italy) 6PRX+59. All plants were hand-picked at full maturation during summer 2023.

3.2. Steam Distillation

The steam distillation of ART, ROS, and LAI was performed using an industrial apparatus equipped with a 250 L boiler (Albrigi Luigi s.r.l., Stallavena, VR, Italy) by Giardino delle Erbe "Rinaldi Ceroni", Casola Valsenio (Ravenna, Italy) farm. The steam distillation of ECHI, HEL, LAV, MEL, SAO, and SAS were performed using an industrial apparatus equipped with a 1500 L boiler (Albrigi Luigi s.r.l., Stallavena, VR, Italy) by the "Officine aromatiche del Frignano" with a 1500 L boiler (Albrigi). Briefly, aerial parts were steam distilled for 1 h and the EO was collected in a Florentine flask and stored at room temperature in an amber glass bottle until the analysis. The oil-exhausted biomass of each plant was collected, air-dried, and stored at room temperature.

3.3. Chemical Characterization of the EOs

The obtained EOs were analyzed by GC to qualitatively and quantitatively determine their chemical composition.

3.3.1. GC-MS Analysis

Analyses were performed on a 7890A gas chromatograph coupled with a 5975C net-work mass spectrometer (GC-MS) (Agilent Technologies, Milan, Italy). Compounds were separated on an Agilent Technologies HP-5 MS cross-linked poly-5% diphenyl–95% dimethyl polysiloxane (30 m × 0.25 mm i.d., 0.25 μm film thickness) capillary column. The column temperature was initially set at 45 °C, then increased at a rate of 2 °C/min up to 100 °C, then raised to 250 °C at a rate of 5 °C/min and finally held for 5 min. The injection volume was 0.1 μL, with a split ratio 1:20. Helium was used as the carrier gas, at a flow rate of 0.7 mL/min. The injector, transfer line, and ion-source temperature were 250, 280, and 230 °C, respectively. MS detection was performed with electron ionization at 70 eV, operating in the full-scan acquisition mode in the m/z range 40-400. The EOs were diluted 1:20 (v/v) with n-hexane before GC-MS analysis.

3.3.2. GC-FID Analysis

Chromatographic characterization of EOs was performed on a 7820 gas chromatograph (Agilent Technologies, Milan, Italy) with a flame ionization detector (FID). EOs and the mixture of aliphatic hydrocarbons (C_8–C_{40}) were diluted 1:20 (v/v) with Hex before GC-FID analysis. Helium was used as

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carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were set at 250 and 300 °C, respectively. EO components were separated on an Agilent Technologies HP-5 crosslinked poly-5% diphenyl–95% dimethylsiloxane (30 m x 0.32 mm i.d., 0.25 µm film thickness) capillary column. The column temperature was initially set at 45 °C, then increased at a rate of 2 °C/min up to 100 °C, then raised to 250 °C at a rate of 5 °C/min, and finally maintained for 5 min. The injection volume was 1 µL, with a split ratio 1:20.

Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic reference standards run under the same conditions and by comparing the linear retention indices (LRIs) relative to C_8–C_{40} n-alkanes obtained on the HP-5 column under the above-mentioned conditions with the literature. Peak enrichment by co-injection with authentic reference compounds was also carried out. A comparison of the MS-fragmentation pattern of the target analytes with those of pure components was performed, by using the National Institute of Standards and Technology mass-spectral database.

The relative percentage of individual components was expressed as the peak area percentage relative to the total peak area obtained from GC-FID analysis. Semi-quantitative data were calculated as the mean of two analyses.

The data acquisition and processing were performed using the OpenLab CDS C.01.04 (Agilent Technologies, Santa Clara, CA) software.

3.4. Preparation of NADES Mixtures

ChCl was selected as the HBA while lactic acid, citric acid, and urea were tested as HBD. The preparation of the NADES formulations was performed according to Bakirtzi et al. [32]. Briefly, a fixed amount of ChCl was mixed with the HBDs at different molar ratios as reported in Table 3. The mixtures were heated under stirring at 50 °C until a transparent and homogeneous liquid was obtained. Afterwards, 20% (v/v) of water was added to the mixtures to reduce the viscosity of the NADES.

Table 3. NADES formulations prepared.

Name	Eutectic mixture	Molar ratio
CCA11	Choline chloride : Citric acid	1 : 1
CCA12	Choline chloride : Citric acid	1 : 2
CLA11	Choline chloride : Lactic acid	1 : 1
CLA12	Choline chloride : Lactic acid	1 : 2

3.5. Oil-Exhausted Biomass Extraction

The exhausted biomasses obtained from the steam distillation were grounded and extracted with both EtOH, as conventional solvent, and different NADES mixtures.

3.5.1. Ethanolic Extraction

Approximately 250 mg of each sample was extracted by ultrasonication with 40 mL of 70% EtOH at room temperature for 15 min. The sample was then centrifuged at 3200 rpm for 5 min, and the supernatant was filtered through paper. The biomass was further extracted with 40 mL, followed by 20 mL of the same solvent, under the same conditions. Finally, the filtrates were combined in a 100 mL volumetric flask, and the solution was stored at -4 °C until analysis. All extractions were performed in triplicate.

3.5.2. NADES Extraction

The NADES extraction was carried out according to Bakirtzi et al. [32]. Briefly, 500 mg of each sample was extracted with 25 mL of NADES under ultrasonication for 90 min at 80 °C. The sample

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was then vacuum-filtered through paper, and the filtrate was diluted with Milli-Q water in a 50 mL volumetric flask.

3.6. Characterization of Biomass Extracts

The EtOH extracts and the optimized NADES extracts of ART, ECHI, HEL, LAV, LAI, MEL, SAO, ROS, and SAS were analyzed by UHPLC-HRMS to determine the polyphenolic composition. Subsequently, the most characteristic compounds were quantified using HPLC-DAD.

3.6.1. UHPLC-HRMS Analysis

The analyses were performed on a Thermo Scientific (Waltham, MA, USA) UHPLC Ultimate 3000, equipped with a binary pump, a vacuum degasser, a thermostated autosampler, a thermostated column compartment, and a Q-Exactive Orbitrap mass spectrometer, with a heated electrospray ionization (HESI) source. An Ascentis Express C_{18} column (100 mm × 2.1 mm I.D., 3 µm, Supelco, Milan, Italy) was used. The mobile phase was composed of (A) 0.1% HCOOH in water and (B) 0.1% HCOOH in ACN, and the gradient elution was set as follows: 0-2 min, 2% B; 2-20 min, 35% B; 20-25 min, 98% B; 25-35 min, 2%. The flow rate was set at 0.4 mL/min and the injection volume was 10 µL. The column temperature was 25 °C.

MS acquisition was performed in negative ionization mode. The source parameters were set as follows: sheath gas (N_2) 45, auxiliary gas (N_2) 25, sweep gas (N_2) 2, auxiliary gas temperature 290 °C, and electrospray voltage 3.80 kV (+) and 3.40 kV (−). A data-dependent acquisition strategy was used to acquire both Full MS and higher energy collisional dissociation (HCD) fragmentation spectra. Mass analyzer acquisition parameters were set as follows: Full MS scan range 100<m/z<100 at 35000 full width half maximum (FWHM) resolving power with an automatic gain control (AGC) target set at 1×10^6 ions with 200 ms maximum injection time; Top 2 HCD fragmentation spectra of most abundant precursor ions were acquired at 17500FWHM resolving power using an isolation window of 1.0 *m*/*z* and stepped normalized collision energy (NCE) at 20, 30 and 50.

3.6.2. HPLC-DAD Analysis

Chromatographic analyses were performed using the Agilent 1260 Infinity II instrument (Agilent Technologies), which includes a quaternary pump (Quaternary Pump 1260), an autosampler (Vialsampler 1260), and a UV/DAD detector (Diode Array WR 1260). Chromatograms were recorded and analyzed using the Agilent Open Lab CDS Version 2.6 software (Agilent Technologies). Chromatographic separation was conducted using two previously developed and validated methods, one for the ECHI samples and the other one for the remaining samples. For both methods, the flow rare was set at 1 mL/min and the injection volume was 10 µL. Before injection, all samples were filtered through a 0.2 µm PTFE (polytetrafluoroethylene) filter and then poured into the vials. All the analyses were carried out in duplicate.

For the ECHI samples, the mobile phase was composed of (A) 0.1% HCOOH in water and (B) ACN, and the gradient elution was set as follows: 0 min, 15% B; 0-10 min, 30% B; 10-18 min, 65% B; 18-25, 80% B; 25-30 min, 90% B. The total run time was 32 min ad the equilibration time was 5 min.

For all the other samples, the mobile phase was composed of (A) 0.3% HAc in water and (B) ACN, and the gradient was set as follows: 0 min, 17% B; 0-35 min, 23% B; 35-52 min, 49% B.

4. Conclusions

The extraction processes of medicinal plants pose significant sustainability challenges, mostly related to the disposal and recovery of the generated biomasses. Indeed, quite often, these biomasses are unused despite containing a high number of bioactive compounds. Among these, the main ones are polyphenols, a big class of compounds widely studied worldwide due to their numerous biological activities, including antioxidants, anti-inflammatory, and antimicrobial properties.

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This study has provided an overview of the method to recover these biomasses using natural deep eutectic mixture solvents, known as NADES. Through advanced analytical techniques like GC-FID, GC-MS, HPLC-DAD, and UHPLC-HRMS, the research has shown that NADES offer a higher extraction capacity compared to conventional organic solvents, such as EtOH. Moreover, NADES exhibit several environmentally friendly characteristics that enhance their sustainability profile, one of them being their biodegradability, reducing their impact on the environment. Additionally, unlike EtOH, NADES are biocompatible and non-toxic, making them safe for biological systems and minimizing risks to human health, animal health, and the environment. For these reasons, NADES could be used for extracting bioactive compounds from biomass for applications in the pharmaceutical, cosmetic, and food industries. Furthermore, their demonstrated antimicrobial activity further expands their potential applications.

Finally, other tests will be conducted to investigate the potential recovery of polyphenols extracted from NADES and the recycling of the eutectic solvent itself, with the purpose of making the process more sustainable.

Looking ahead, future research should continue to explore NADES in the context of sustainability. Further investigations should focus on their environmental impact throughout their lifecycle and their potential to support circular economy principles. Additionally, it is essential to evaluate potential formulations containing NADES extracts from aromatic plant biomasses for applications in the pharmaceutical, nutraceutical, and cosmetic industries.

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Abbreviations

The following abbreviations are used in this manuscript:

EO	Essential oil
NADES	Natural Deep Eutectic Solvents
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donator
ART	*Artemisia dracunculus*
ECHI	*Echinacea purpurea*
HEL	*Helichrysum italicum*
LAV	*Lavandula angustifolia*
LAI	*Lavandula x intermedia*

MEL	*Melissa officinalis*
SAO	*Salvia officinalis*
ROS	*Salvia rosmarinus*
SAS	*Salvia sclarea*
GC	Gas chromatography
UHPLC-HRMS	Ultrahigh-Performance Liquid Chromatography–High-Resolution Mass Spectrometry
HPLC-DAD	High-Performance Liquid Chromatography with Diode Array Detection
LRI	Linear retention index
EtOH	Ethanol
Hex	hexane
ChCl	Choline chloride

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