ASSESSMENT OF THE USE OF COMMON JUNIPER (*Juniperus communis* L.) FOLIAGE FOLLOWING THE CASCADE PRINCIPLE: ESSENTIAL OIL, ABSORBENTS AND BIOCHAR

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ABSTRACT: The main objective of the BeonNAT project is to create added-value bio-based products by growing underused tree and shrub species in marginal land. One of the species selected within the project is juniper (*Juniperus communis* L). Foliage biomass coming from a natural population in Spain was selected and a first sampling of wild biomass (1000 kg fresh plants with stem diameters below 50 mm) was performed. This biomass was distilled and, after it, different alternatives were defined in the project to obtain bioproducts from the distilled biomass following the cascade concept. Among them, absorbents for the pet industry and biochar have been selected in this work.
Concerning the steam distillation, an essential oil yield of 0.44% (w/w d.b) was obtained and the characterisation of the essential oil compounds showed that it was close to meet the limits considered by the ISO 8897:2010. A fraction of the distilled biomass was used to produce pellets, whose quality as absorbents for the pet industry was promising. Finally, the rest of the distilled biomass was used to obtain biochar with a yield of 28.79 % (w/w d.b) and characteristics which indicated a good degree of carbonisation and stability.
Keywords: *Juniperus communis* L., biobased products, essential oil, biochar, pellet, absorption

1 INTRODUCTION

A sustainable and circular European bioeconomy is needed to deal with global challenges like climate change, land and ecosystem degradation and a growing population. In this sense, modernisation and strengthening of the EU industrial base through the creation of new value chains and greener, more cost-effective industrial processes are of particular importance [1]. However, the production of new bio-based products from vegetal biomass entails the use of land, which is a finite and scarce resource, and competition with food production or other necessities such as preservation of habitats, regeneration of ecosystems or sequestration of carbon, can happen [2]. Then, the production of biomass on abandoned, unused or severely degraded land (also referred to as marginal land) [3] could be a way to overcome a wide range of land-use challenges [2].

The main objective of the BeonNAT project is to create added-value bio-based products growing underused tree and shrub species in marginal land. One of the species selected within the project is *Juniperus communis* L. It is an evergreen, perennial, long-lived coniferous plant which has the largest range of any woody plant in the cool temperate geographical regions of Northern Hemisphere, from the Arctic south, in mountains, to around a latitude of 30º north in Europe, Asia and North America [4].

Nowadays, the berry cones of *J. communis* and their essential oils are recognised by the European Pharmacopoeia and the essential oil of these berry cones has demonstrated to have strong antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory properties [4]. Some differences have been found between the essential oil of *J. communis* berry cones and leaves with regard to essential oil yield and composition [4-6], and most of the studies related to the bioactivity of *J. communis* essential oils has been focused on the oil coming from the berry cones. However, some studies have shown antioxidant and insecticidal properties for the essential oil obtained from the foliage of this species [7, 8], but more research work is needed to study the bioactivity of this essential oil.

After the steam distillation process, different by-products are obtained, for example the distilled vegetal biomass, which is used as fuel to produce process heat or for composting in some industrial installations. However, other uses, like the extracts [9-11] or bio-oil [12] production are being investigated with different species. To the authors' knowledge, alternative uses of distilled *J. communis* have not been studied.

The objective of this work is to assess the yield and quality of different products obtained in the *Juniperus communis* value chain, such as essential oil, biochar and absorbents, following the cascade principle.

2 MATERIALS AND METHODS

2.1 Vegetal material

A natural population of *Juniperus communis* located in a mountain area in Spain (UTM coordinates 30T 545081; 4649553) was selected and a sample about 1000 kg of foliage coming from plants with stem diameter below 50 mm was collected.

Figure 1: Sampling of *Juniperus communis*

2.2 Conditioning of biomass

After the collection, the foliage biomass was crushed to a size of 20 mm by means of a shredder and afterwards, it was distilled using a steam distillation pilot plant. The distilled biomass was air-dried at temperature below 40 ºC and was separated into two fractions using a sieving and blowing step. The sieve used had a mesh of 4 mm and a fine fraction composed by the particles with size below 4 mm and blown particles and a coarse fraction composed by the particles with a size above 4 mm were obtained. Finally, the coarse fraction was milled in a hammer mill at 8 mm. Subsamples were taken along the process to determine the moisture content in a oven at 105 ºC following the standard ISO 18134-2:2017. The conditioning process was carried out at CEDER-CIEMAT facilities. Figure 2 shows the different steps followed during the conditioning process.

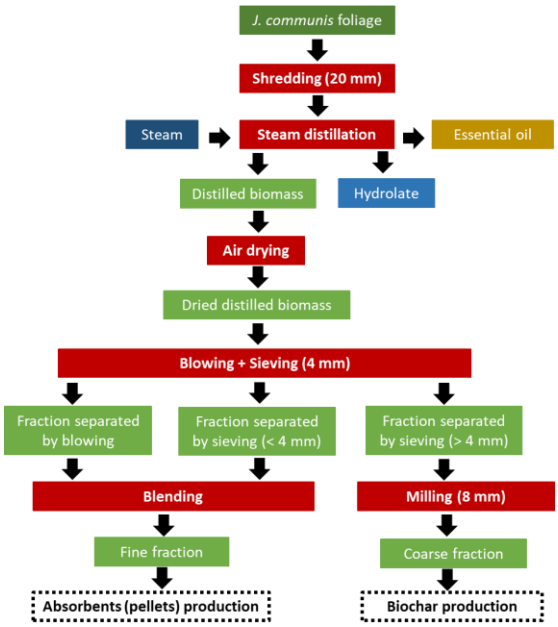

Figure 2: Block diagram of the biomass conditioning process and cascading

2.3 Steam distillation and essential oil characterisation

A steam distillation pilot plant located at CEDER-CIEMAT was used to distil the *J. communis* biomass (Figure 3). This plant is composed by a 1.8 m^3 stainless steel still, an electric boiler to produce the steam (0.5 barg, 25 kg h^{-1}), a cooling system for steam condensation and a glass Florentine flask to separate the hydrolate and the essential oil by density. Batch distillations were performed with two repetitions of 400 kg each per sample and a distillation duration of 6 h. Time was measured from the moment the first drop of distillate fell. The essential oil samples were dried using anhydrous sodium sulphate and, after filtration, they were weighed and stored at 4 ºC until further analysis. The oil yield for each sample was calculated as a percentage (w/w) on a biomass dry weight basis.

One sample of essential oil was obtained blending the two samples of essential oil produced in the steam distillation and it was analysed by Gas Chromatography - Mass Spectrometry (GC-MS) on a Shimadzu GC-2010 Plus chromatograph equipped with AOC-20iPlus automatic injector (Shimadzu) and a SH-RXi fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; Shimadzu, USA). The oven temperature program was as follows: 40 ºC for 4 min, raised at 3 ºC/min to 175 ºC, then at 15 ºC/min to 300 ◦C and held for 10 min. The injector temperature was set at 260 ºC, with a transfer line at 280 ◦C and an ion source at 220 ºC. The ionisation energy was 70 eV, and a scan range of 35–500 u with a scan time of 0.3 s was used. The essential oil was diluted in HPLC grade n-hexane (1:100) and 1 µL was injected with a split ratio of 1:10. Identification of compounds was assigned by matching their mass spectra with NIST17 data and by determining the linear retention index (LRI) based on the linear retention index obtained with a mixture of n-alkanes (C8–C40, ref. 40147-U, Supelco) analysed under identical conditions. Quantification was performed using the relative peak area values obtained directly from the total ion current (TIC) values, and the results were expressed as the relative percentage of total volatiles.

Figure 3: Steam distillation pilot plant located at CEDER-CIEMAT (left) and detail of the Florentine flask

2.4 Pyrolysis tests and biochar characterisation

The coarse fraction obtained after the conditioning of the distilled biomass (Figure 2) was pyrolysed using an innovative pilot plant composed by a continuous auger type pyrolysis reactor (SPYRO) and by an in-series condensation unit (Figure 4). SPYRO is designed to process up to 3 kg h^{-1} of biomass and can be operated up to 600°C. The rotating speed of the reactor screw can be easily adjusted to vary the solids residence time, from few minutes up to 1 hour. A total of 4220 g of wet feedstock were processed with an average heating rate of 17.80 °C min^{-1} and a residence time of about 30 minutes at the maximum pyrolysis process temperature of 550 °C. The charcoal is collected by gravity in a sealed vessel, while volatiles are separated into a pyrolysis liquid that is collected in glass bottles, and an off-gas stream composed of permanent gas.

Figure 4: Pyrolysis pilot plant located at RE-CORD

Biochar obtained was characterised for the following parameters: moisture content, ash content, volatile matter content, fixed carbon, CHN elemental analysis and water

holding capacity at 0.01 bar.

Moisture, ash content and volatile matter were analysed using a Leco Thermogravimetric Analyser TGA 701. Moisture analysis was measured at 105 °C following the UNI EN ISO 18134-3 standard, the ash content was measured at 550 °C according to UNI EN ISO 18122, the volatile matter at 900 °C according to UNI EN ISO 18123. The fixed carbon was calculated according to UNI EN 1860-2. The total carbon (C), hydrogen (H), nitrogen (N) contents were analysed in a Leco TruSpec CHN analyser according to UNI EN ISO 16948. Water holding capacity was determined according to Italian D.M. 01/08/1997 Method 5.

2.5 Pelletisation tests and absorbent characterisation

Finally, the fine fraction obtained after the biomass conditioning (Figure 2) was used to produce pellets in a small pilot plant located at CEDER-CIEMAT (Figure 5). The pellet press, KAHL 14-175, is a flat-die machine with dosing hopper and variable speed. With an engine of 3 kW, this press can produce 20-40 kg pellets h^{-1}. The flat die used has a diameter of 175 mm, holes with 6 mm of diameter and a die compression (length of the inlet cone + straight channel of the hole) of 28 mm. Two rollers with a width of 15 mm and rotating speed between 0.5 and 0.8 m s^{-1} force the material to go through the flat die.

Figure 5: Small pilot plant located at CEDER-CIEMAT

At the beginning of the pelletisation tests, water and biomass flows fed to the pellet press were modified as the process went on, with the aim of optimising the operation of the machine (i.e. stable power demand and low vibration) and obtaining high quality pellets. When these conditions were reached, it was considered that steady state had been achieved and it was maintained for 40 minutes. Once the pellets were cooled down, they were manually sieved with a 3.15 mm sieve, in order to simulate an industrial process

The process variables recorded in the pelletisation tests were the specific mass flow (in kg of dry matter per hour and per kW of drive power) and the specific energy (in kWh per t of dry matter). The specific mass flow is calculated as the mass of pelletised material (in kg of dry matter) divided by the time utilised to pelletise it (in hours) and by the power of the pellet press (3 kW). The specific energy is calculated as the active electric energy demanded by the different items to pelletise the material, divided by the mass of pellets obtained (in t of dry matter). The water absorption capacity of the obtained pellets was analysed at TOLSA facilities, following the standard UNG-008, to determine their quality as absorbent cat litter. It is an important cat litter property since liquid saturation of the particles generates changes in the litter performance. It is determined by means of the Westinghouse method, which tests the static water absorption capacity of the vegetal pellets when fully immersed and wet until saturation and/or breakup. It provides information on the absorbent behaviour and capacity of the material in liquid saturation cases, as well as its degradation.

3 RESULTS AND DISCUSSION

3.1 Vegetal material

1,050 kg of *J. communis* foliage coming from plants with stem diameter below 50 mm were collected in April 2021. Taking into account that this is a dioecious plant, 50% of the biomass material corresponded to male plants and 50% to female plants.

3.2 Conditioning of biomass and steam distillation

All the biomass collected was shredded at 20 mm and a sample was separated in order to analyse its moisture content, which was 42.5%.

Two samples of 400 kg of shredded material were distilled in the pilot plant described in section 2.3 and the average essential oil yield was 0.44% (standard deviation: 0.037), expressed in weight percentage of dry essential oil referred to dry juniper. This value was of the same order of magnitude as those obtained in a previous work with *J. communis* collected in May 2018 and June 2020 in an area next to the area considered in this work and distilled in a plant with a capacity of 30 L [13]. However, the yields reported for *J. communis* foliage distillation in other studies are variable, between 0.05 and 2.43%, although these results correspond to tests carried out on a laboratory scale and using a Clevenger apparatus with steam distillation or hydrodestillation [14-18].

After the distillation, the distilled biomass was air-dried, achieving a moisture content of 10.3%, and was separated into two fractions using a sieving (4 mm) and blowing step. Two tests of separation with 200 kg of air dried distilled biomass each were carried out obtaining the following results, expressed as average value in weight percentage:

- Biomass from blowing: 11.9% (standard deviation: 0.42).
- Biomass < 4 mm from sieving: 54.6% (standard deviation: 1.04).
- Biomass > 4 mm from sieving: 33.5% (standard deviation: 1.46)

Consequently, the fine fraction composed by the biomass from blowing and the fine biomass obtained after sieving corresponded to 66.5% and the coarse fraction corresponded to 33.5% of the total biomass distilled.

Considering the cascade defined in this work (Figure 2), the fine fraction was pelletised and the coarse fraction was milled at 8 mm previously to be pyrolysed.

3.3 Essential oil composition

The essential oils obtained in the two steam distillation tests were blended and analysed using GC-MS. The components identified and their quantification using the relative area percentage are shown in Table I.

The characterisation of the essential oil showed that its composition was close to meeting the limits considered by the ISO 8897:2010 "Oil of juniper berries (*Juniperus communis* L.)" for most compounds except limonene, which was higher than the limit, and α-pinene, that was slightly below it. The main compounds (>5%) were α-pinene (18%), limonene (16%), sabinene (11%), myrcene (5.9%) and *cis*-thujopsene (5.1%).

Table I: Chemical composition of the *J. communis* essential oil

Biomass Sources	Retention time (min)	LRI[a]	LRI[b]	Relative (%)[c]
Tricyclene	13.39	915	921	0.071±0.002
α-Thujene	13.70	921	924	2.11±0.04
α-Pinene	14.05	928	932	18±2
Camphene	14.76	942	946	0.269±0.004
Sabinene	16.11	968	969	11±1
β-Pinene	16.24	970	974	1.69±0.02
Myrcene	17.08	986	988	5.9±0.4
2-Carene	17.51	995	1001	0.36±0.03
α-Phellandrene	17.70	998	1002	2.2±0.1
3-Carene	18.00	1004	1008	0.61±0.04
α-Terpinene	18.34	1011	1014	2.3±0.1
o-Cymene	18.76	1018	1022	1.9±0.1
Limonene	19.00	1023	1025	16±2
cis-ocimene	19.51	1033	1032	0.014±0.002
β-Ocymene	20.04	1043	1044	0.12±0.01
Isopentyl butanoate	20.47	1052	1052	0.073±0.002
γ-Terpinene	20.56	1053	1054	3.4±0.1
cis-Sabinene hydrate	20.97	1061	1065	0.043±0.002
Terpinolene	22.08	1083	1086	2.2±0.2
Isopentyl isovalerate	22.98	1100	1102	0.079±0.003
β-thujone	23.50	1111	1112	0.0599±0.0001
cis-p-Menth-2-en-1-ol	23.74	1115	1118	0.075±0.001
α-Campholenal	23.98	1120	1122	0.134±0.004
trans-Pinocarveol	24.63	1133	1135	0.17±0.01
Terpinen-4-ol	26.54	1172	1174	3.6±0.1
α-Terpineol	27.19	1185	1186	0.41±0.04
Myrtenol	27.47	1191	1194	0.09±0.01
trans-Carveol	28.56	1213	1215	0.03±0.01
Citronellol	28.99	1222	1223	0.09±0.01
Cumin aldehyde	29.54	1234	1238	0.04±0.01
Carvacrol methyl ether	29.74	1238	1241	0.15±0.03
Isoamyl hexanoate	30.01	1244	1246	0.017±0.003
Phellandral	31.18	1269	1273	0.15±0.02
α-Terpinen-7-al	31.64	1279	1283	0.01±0.01
Bornyl acetate	31.72	1281	1284	0.29±0.05
2-undecanone	32.03	1287	1293	0.043±0.002
Myrtenyl acetate	33.51	1320	1324	0.04±0.01
α-Terpinyl acetate	34.59	1345	1341	0.34±0.01
α-Cubebene	34.62	1345	1345	0.6±0.2
α-Ylangene	35.62	1368	1373	0.02±0.01
α-Copaene	35.81	1372	1374	0.4±0.1
Isolongifolene	36.40	1386	1389	0.044±0.003
β-Elemene	36.50	1388	1389	1.2±0.2
Longifolene	37.13	1402	1407	0.25±0.05
β-Funebrene	37.43	1410	1413	0.29±0.03
β-Caryophyllene	37.72	1416	1417	2.2±0.3
cis-Thujopsene	38.22	1428	1429	5.1±0.5
γ-Elemene	38.41	1433	1434	0.021±0.003
Aromadendrene	38.53	1436	1439	0.025±0.004
cis-muurola-3,5-diene	39.00	1447	1448	0.13±0.02
Humulene	39.15	1451	1452	1.5±0.2
cis-Muurola-4(15),5-diene	39.54	1460	1465	0.06±0.01
Cadina-1(6),4-diene	39.96	1470	1475	0.2±0.03
γ-Muurolene	40.07	1473	1478	0.5±0.1
Germacrene D	40.28	1478	1480	1.8±0.3
β-Eudesmene	40.51	1483	1489	0.5±0.1
α-Muurolene	41.05	1496	1500	1.0±0.2
Cuparene	41.29	1502	1504	0.48±0.02
β-Curcumene	41.46	1506	1509	0.2±0.1
γ-Cadinene	41.62	1511	1513	1.6±0.3
β-Cadinene	41.97	1520	1518	1.7±0.3
Germacrene B	43.36	1555	1559	1.6±0.3
Nerolidol	43.45	1557	1561	0.11±0.04
Spatulenol	44.14	1575	1577	0.07±0.02

Table I: Chemical composition of the *J. communis* essential oil (cont.)

Biomass Sources	Retention time (min)	LRI[a]	LRI[b]	Relative (%)[c]
Caryophyllene oxide	44.38	1581	1582	0.19±0.03
Cedrol	45.11	1599	1600	0.12±0.01
Humulene epoxide II	45.39	1607	1608	0.10±0.01
tau-Cadinol	46.55	1638	1638	0.14±0.05
tau-Muurolol	47.06	1651	1640	0.09±0.01
Total identified				96.4%
Monoterpene hydrocarbons				68.1%
Oxygen-containing monoterpenes				5.7%
Sesquiterpene hydrocarbons				21.5%
Oxygen-containing sesquiterpenes				0.8%
Esters				0.2%
Not identified				3.6%

[a]LRI. linear retention index determined on a DB-5 MS fused silica column relative to a series of n-alkanes (C8–C40). [b]LRI. linear retention index reported in the literature [19]. [c] relative % is given as average ± standard deviation.

3.4 Pyrolysis tests and biochar characterisation

The coarse fraction obtained after the separation of distilled juniper biomass was pyrolysed in the RE-CORD pilot scale plant and a biochar yield of 28.79 % by dry weight was obtained. The biochar produced showed a total carbon content of 87.8 % w/w dry basis and a fixed carbon content of 78.4 % w/w dry basis. As reported by Leng et al. [20], biochar fixed carbon is closely related to stable C content. Moisture content was 1.8 w/w wet basis, volatile matter and ash content resulted respectively in 13.0 and 8.7 % w/w dry basis.

Furthermore, the molar H/C ratio, that is one of the most important characterising features of biochar and is indispensable for the determination of the C-sink value, resulted in 0.35. Fixed carbon content and H/C molar ratio indicate a good degree of carbonisation and stability for the biochar obtained [20, 21]. The biochar water holding capacity test at 0.01 bar resulted in 141.6 % w/w wet basis.

3.5 Pelletisation tests and absorbent characterisaton

The fine fraction obtained after the separation of distilled juniper biomass (moisture content: 12.8%) was pelletised, with a specific mass flow of 7.6 kg of dry matter per hour and per kW of drive power and a specific energy of 78 kWh per t of dry matter. Comparing these values with those obtained in previous works to produce solid biofuels, the specific mass flow was 27% higher than the values obtained in the pelletisation of shrub biomass (*Genista cinerascens* L.) [22] and pine sawdust [23], while the specific energy was 34% lower.

The water absorption capacity of the pellets obtained was 267%, which can be considered as appropriate to produce absorbents for the pet industry, according to the current requirements demanded by the consumer in the absorbent litter market.

4 CONCLUSIONS

The biomass of *Juniperus communis* coming from clearing in a natural population located in Spain could be used as raw material to produce essential oil, biochar and pet absorbents within the cascade concept. Although further investigation is needed to characterise the whole chain and products, preliminary tests show that essential oil is obtained with a yield of 0.44% and the main composition is 18% α-pinene, 16% limonene, 11% sabinene, 5.9% myrcene and 5.1% *cis*-thujopsene. Moreover, the distilled biomass can be divided into two fractions appropriate to produce biochar and absorbents for the pet industry, with promising results regarding both yield and quality.

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7 LOGO SPACE


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