Ayben Kilic-Pekgözlü, Esra Ceylan, Ayhan Gencer, Rıfat Kurt[1]

Optimization for Green Path in Wood Extractives by Taguchi Analysis

Primjena Taguchijeve analize za optimizaciju procesa ekstrakcije drva na načelima zelene kemije

ORIGINAL SCIENTIFIC PAPER

Izvorni znanstveni rad

Received – prispjelo: 29. 9. 2021.

Accepted – prihvaćeno: 23. 3. 2022.

UDK: 630*81; 674.032.475.4

https://doi.org/10.5552/drvind.2022.2137

ABSTRACT • *Extractive composition of Pinus pinaster (Maritime pine) wood was studied with choline chloride based deep eutectic solvents (DES). Two different eutectic mixtures (ChCl: Et-Gly; ChCl:Urea 1:2 molar ratio), two different extraction methods (hot water bath, ultrasound assisted extraction), and temperature (40 – 60 °C), time (30 – 60 min.) and solid:liquid ratio (1:10 – 1:20 g/mL) parameters were applied. For the optimization of operation conditions, Taguchi analysis was performed. Resin acids formed the chemical composition of Pinus pinaster (Maritime pine) wood. Dehydroabietic acid, abietic acid and isopimaric acid were found to be the major compounds. The impact of parameters on the performance of the system was determined as follows: DES type > solid:liquid ratio > extraction method > temperature > time. So, for the extraction of lipophilic compounds in Maritime pine wood, the optimum conditions were determined as hot water bath extraction at 60 °C with ethylene glycol and 1:10 solid liquid ratio for 60 min.*

KEYWORDS: *deep eutectic solvent; Pinus pinaster; resin acids; choline chloride; ethylene glycol; urea; Taguchi analysis*

SAŽETAK • *U radu su istražene ekstraktivne tvari drva primorskog bora (Pinus pinaster) izolirane primjenom eutektičkog otapala (DES) na bazi kolin klorida. Pritom su primijenjene dvije eutektičke mješavine (ChCl: Et-Gly i ChCl: Urea, molarni omjer 1:2) i dvije metode ekstrakcije (vrućom vodom i uz primjenu ultrazvuka), pri temperaturi 40 i 60 °C i s vremenom ekstrakcije od 30 i 60 minuta, uz varijabilni odnos kruto – tekuće 1 : 10 i 1 : 20 g/mL. Za optimizaciju procesa ekstrakcije primijenjena je Taguchijeva analiza. Rezultati istraživanja pokazuju da se ekstraktivne tvari drva primorskog bora (Pinus pinaster) uglavnom sastoje od smolnih kiselina s dehidroabietinskom, abietinskom i izopimarnom kiselinom kao glavnim spojevima. Usto je utvrđen i utjecaj pojedinog parametra na efikasnost procesa ekstrakcije kako slijedi (rangirano od onoga s najvećim prema onome s najmanjim utjecajem): vrsta eutektičkog otapala > odnos kruto – tekuće > metoda ekstrakcije > temperatura ekstrakcije > vrijeme ekstrakcije. Na temelju analize dobivenih podataka određeni su optimalni parametri za ekstrakciju drva primorskog bora. To su: ekstrakcija vrućom vodom pri 60 °C, uz upotrebu etilen glikola i omjer kruto – tekuće 1:10 tijekom 60 minuta.*

KLJUČNE RIJEČI: *eutektička otapala; Pinus pinaster; smolne kiseline; kolin klorid; etilen glikol; urea; Taguchijeva analiza*

[1] Authors are researchers at Bartın University, Forestry Faculty, Forest Industry Engineering, Bartın, Turkey. https://orcid.org/0000-0002-3640-6190, https://orcid.org/0000-0002-0758-5131, https://orcid.org/0000-0002-7136-7665

1 INTRODUCTION
1. UVOD

In the concept of "green chemistry", reduction of unsafety and petroleum based solvent use in industry becomes a priority for EU between 2010-2050 (Bubalo *et al.,* 2015). Different solvents, e.g. supercritical-subcritical solvents, ionic liquids (ILs), deep eutectic solvents (DES), low-melting mixtures (LMMs), are classified as safe and non-hazardous solvents (Fischer, 2015).

DES, consisting of two or more compounds, are formed from hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA). DES are negligibly volatile, cheap, non-toxic, low-flammable and thermally stable, often biodegradable, and not requiring purification (Ozturk *et al.,* 2018a). They are classified into four groups: Type I (organic salts + metal salts), Type II (organic salts + metal hydrates), Type III (organic salts + HBD) and Type IV (metal chlorides + HBD). Type III deep eutectic solvents, used in this study, are applied in fractionation of lignocellulosic biomass, biodiesel production, metal processing and extraction of polar molecules and bioactive compounds. They are also used in pharmaceutical and biomedical applications (Ozturk *et al*., 2018a; Cao *et al*., 2018; Meng *et al*., 2018; Zdanowicz *et al*., 2018; Barbieria *et al*., 2020). Choline choride (ChCl), a non-toxic and biodegrable compound, is mostly used in the DES mixtures as a HBD. With these features, ChCl based DES solvents are convenient for the pharmaceutical and cosmetic use (Häkkinen, 2020).

Extractives, defined as low molecular compounds in the woody plants, are composed of different chemical substances. These compounds can be classified into two groups - lipophilics and hydrophilics (Sjöström, 1981). For the extraction of lipophilic compounds, non-polar solvents are used, while polar solvents are used for hydrophilics (Vek *et al*., 2020). Although the amount of extractives is less than 10 % of dry wood, they are used in different areas, e.g lipophilics in pharmacy, food and cosmetic industries. Hydrophilic compounds have antioxidant, antimicrobial, antiviral, cancerogenic and cardio protective effects (Fengel and Wegener, 2003; Benouadah *et al*., 2018). Because of these features, extractives are becoming increasingly important. The use of non-toxic, biodegradable chemicals has also become popular in the last years.

Pinus pinaster Aiton occurs naturally in Southwest Europe (e.g Spain, Portugal), Western Mediterranean and Northwest Africa as a fast growing species. This species was first planted in 1881 in different regions of Turkey and today it covers a total of 57,378.4 ha (Velioglu *et al*., 2020; Koch, 1972).

It is mainly used in pulp and paper industry both in Turkey and in Europe. It is also used in particleboard and packaging industries. Due to its high resin content, special resin production sites have been created for *Pinus pinaster* in different countries (e.g Portugal, Spain, France, Italy). The yield of resin is 1457-2500 g/tree with acid-paste method in Turkey (Aydin, 2017). It is reported that the turpentine part of this resin is used as antiseptic, diuretic and anthelmintic. Also, local people use the cone extracts of this species to prevent bronchitis and cough (Kurtca and Tumen, 2020). There are several studies regarding the extractives of *Pinus pinaster.* In the wood part, simple phenolics, stilbenes, lignans, flavonoids, organic acids, steryl esters and triglycerides are found to be the major extracts in the hot water aliquot (Conde *et al*., 2014). However, in the bark part, diterpenic compounds that have nutraceutical effects are found to be the main compounds with fatty acids, long-chain alcohols and sterols (Sousa *et al*., 2018).

In this study, a new generation of choline chloride based on two different deep eutectic solvents was used to determine the extractive composition of *Pinus pinaster* wood. Different temperatures (40-60 °C), time (30-60 min.), solid:liquid ratio (1:10-1:20 g/mL) and extraction methods (hot water bath- ultrasound assisted extraction) were used. For the optimization of operation conditions, Taguchi method was performed. Although there are studies about the phenolic compositions of wood with deep eutectic solvents, lipophlics are studied for the first time.

2 MATERIALS AND METHODS
2. MATERIJALI I METODE

2.1 Materials
2.1. Materijal

In this study, *Pinus pinaster,* obtained from Zonguldak –Turkey, was used as wood material. Bark was removed before chipping the woody parts into small matchstick size. Samples were grounded in a Wiley mill and freeze-dried before the extraction. Choline chloride, urea and ethylene glycol were purchased from Merck in analytical grades and used without further purification.

2.2 Deep eutectic solvents (DES) preparation
2.2. Priprema eutektičkih otapala (DES)

Two different DES mixtures were prepared. Molar ratios are listed in Table 1. Mixing procedure was performed at 80 °C until a homogenous clear liquid was obtained.

2.3 Extraction methods
2.3. Metode ekstrakcije

Two different extraction procedures were applied. As a control, sequential extraction with n-hexane and

Table 1 Molar ratios of deep eutectic solvents
Tablica 1. Molarni odnosi eutektičkih otapala

HBA	HBD	Abbreviation *Skraćenica*	Molar ratio (wt/wt) *Molarni omjer* (m/m)
Choline chloride / *kolin klorid*	Urea / *urea*	ChCl : Ur	1 : 2
Choline chloride / *kolin klorid*	Ethylene glycol / *etilen glikol*	ChCl : Et-Gly	1 : 2

acetone:water (95:5 v/v) was carried out for 6 hours in a soxhlet apparatus. With DES mixtures, hot water extraction (HW) and ultrasound assisted extraction (UAE) were performed. Wood samples and DES were put in a test tube and extracted according to the below conditions. After each extraction method, samples were centrifuged for 15 min. at 4000 rpm. 1 mL of aliquot was taken to a new test tube and extracted (liquid-liquid) with acetone before silylation. For the optimization of extraction, some parameters were tested: type of DES (Table 1), solid:liquid ratio (1:10 -1:20 g/mL), temperature (40 – 60 °C) and extraction time (30 – 60 min.). Three repetitions were made for each parameter.

2.4 FID-GC and GC-MS analysis
2.4. Analiza FID-GC i GC-MS

Shimadzu GCMS-QP2010 GC-MS equipped with TRB-5MS column (30 m × 0.25 mm (0.25 µm thickness)) was used for the identification. Temperature program was 120 °C for 1 min. then raised to 310 °C with a 6 °C/min. waiting for 20 minutes. The injection temperature was 260 °C, split ratio was 1:25, ion source was 200 °C and ionization energy 70eV. Wiley and NIST libraries were used. For quantitative analysis, Shimadzu GC 2010 FID-GC was used.

2.5 Taguchi Design
2.5. Dizajn Taguchijeve metode

Taguchi is an effective statistical method to optimize the operation condition settings. With this method, the number of experiments was reduced and so were the costs and time (Kumar *et al*., 2015; Uslu and Aydin, 2020). The method consists of an orthogonal array, signal-to-noise ratio (S/N or SNR), response table and graph (Main Effect Analysis) (Ozakin and Kaya, 2020). To start with Taguchi, operation parameters, quality characteristics and orthogonal array are selected for designing and doing the experiments. Then, the results are analyzed by using signal-to-noise ratio (S/N). Finally, optimum parameters are obtained with the analysis results (Sun *et al*., 2013; Liu *et al*., 2019).

2.5.1 Selection of parameter levels and orthogonal array of Taguchi
2.5.1. Odabir razina parametara i ortogonalni niz Taguchijeve metode

The parameters, affecting the amount of selected compounds (abietic acid, dehydroabietic acid and isopimaric acid, the most abundant compounds), were determined as extraction method, extraction temperature, extraction time and solid/liquid ratio. Table 2 illustrates the factors considered and their levels.

Table 2 Parameters and their levels
Tablica 2. Parametri i njihove razine

Codes *Oznake*	Parameters *Parametri*	Levels / *Razine* 1	Levels / *Razine* 2
A	Method / *metoda*	HW	UAE
B	Time, min / *vrijeme,* min	30	60
C	Temperature, °C *temperatura, °C*	40	60
D	DES	Et-Gly	Ur
E	Solid/Liquid ratio, g/mL *odnos kruto – tekuće,* g/mL	10	20

According to the number of selected parameters and their levels, the L_{32} (2^5) orthogonal array of Taguchi was selected, as shown in Table 3. The main feature of the orthogonal indices is that all the factors are included in the experiment with an equal number of trials.

2.5.2 Signal to noise ratio
2.5.2. Omjer signala i šuma

In the Taguchi method, generally, the S/N is adopted as the indicator of quality (Jiang *et al*., 2020). It is defined as undesired random noise value, desirable signal ratio and shows the quality characteristics of experimental data (Kurt *et. al*., 2009; Gunay *et al*., 2011; Gunay and Yucel, 2013). Depending on the particular characteristics of the design problem, different S/N ratios may be applicable, including "lower is better", "nominal is best", or "higher is better" (Chen *et al*., 2007; Kurt and Can, 2021). In this study, for the calculation of S/N ratio "higher-is-better" performance character was preferred as shown in Eq. 1.

$$S/N = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \tag{1}$$

n is the number of observations of the experiment and y_i is the observed data at the i^{th} experiment (Taguchi *et al*., 2005).

2.5.3 Grey relations analysis
2.5.3. Siva relacijska analiza

In order to verify the Taguchi results and specify the best experiment conditions, Grey relation analysis (GRA) was done. GRA is a part of a system theory, improved for solving complex relationships between alternatives and variables (Yang and Chen, 2006). In case of many criteria and alternatives, this method is

Table 3 Orthogonal array of Taguchi L_{32} (2^5)
Tablica 3. Ortogonalni niz Taguchijeve metode L_{32} (2^5)

Experiment No *Broj eksperimenta*	A	B	C	D	E	Experiment No *Broj eksperimenta*	A	B	C	D	E
1	1	1	1	1	1	17	2	1	1	1	1
2	1	1	1	1	2	18	2	1	1	1	2
3	1	1	1	2	1	19	2	1	1	2	1
4	1	1	1	2	2	20	2	1	1	2	2
5	1	1	2	1	1	21	2	1	2	1	1
6	1	1	2	1	2	22	2	1	2	1	2
7	1	1	2	2	1	23	2	1	2	2	1
8	1	1	2	2	2	24	2	1	2	2	2
9	1	2	1	1	1	25	2	2	1	1	1
10	1	2	1	1	2	26	2	2	1	1	2
11	1	2	1	2	1	27	2	2	1	2	1
12	1	2	1	2	2	28	2	2	1	2	2
13	1	2	2	1	1	29	2	2	2	1	1
14	1	2	2	1	2	30	2	2	2	1	2
15	1	2	2	2	1	31	2	2	2	2	1
16	1	2	2	2	2	32	2	2	2	2	2

commonly used for alignment or streaming of options and choice between alternatives. GRA consist of following steps (Tosun, 2005; Haq *et. al*., 2008; Shi *et. al*., 2015; Panda *et. al*., 2016).

Step 1. Form the decision matrix and uniform the data in order to prohibit unit variations. It is actually necessary because variations between data can be different. Reproduce a value to form the array between 0 to 1 from original value. Three different equation "higher is better", "lower is better" and "nominal is best" are used according to the problem in the normalization process. In this study, "higher-is-better" performance character was preferred as shown in Eq. 2.

$$x_i^*(k) = \frac{x_i^0(k) - min\, x_i^0(k)}{max\, x_i^0(k) - min\, x_i^0(k)} \tag{2}$$

Where $x_i^*(k)$ specifies the sequence after data preprocessing, $x_i^0(k)$ is the measured results, $min\, x_i^0(k)$ is the minimum value $x_i^*(k)$, and $max\, x_i^0(k)$ is the maximum value of $x_i^0(k)$, i is the number of experiments, and k represents the measurement values.

Step 2. Calculation of grey relational coefficient:

$$\xi_i(k) = \frac{\Delta_{min} - \xi\Delta_{max}}{\Delta_{0i}(k) + \xi\Delta_{max}} \tag{3}$$

Where, Δ_{0i} is the deviation sequence of the reference sequence and comparability sequence, Δ_{min} is the minimum value in the sequence, Δ_{max} is the maximum value in the sequence. ξ is defined as identification coefficient and the range is between 0 to 1. Generally, the value of ξ is taken as 0.5.

Step 3. Calculation of grey relational grade is defined as final step. It is calculated according to Eq. 4 averaging the sum of the grey relational coefficients

$$\gamma_i = \frac{1}{n}\sum_{k=1}^{n}\xi_i(k) \tag{4}$$

Where γ_i changes in the range of 0 to 1, and n is the number of experiments. The higher grey relational grade signifies more ideal results.

3 RESULTS AND DISCUSSION
3. REZULTATI I RASPRAVA

3.1 Chemical composition
3.1. Kemijski sastav

The amount of lipophilic compounds after the sequential extraction with n-hexane and acetone are given in Table 4. As seen, it is mainly formed from resin acids and the main compounds are abietic, dehydroabietic and levopimaric acids. In addition, oxidized resin acids (6 %) were found in the n-hexane extract.

Two types of resin acids (Abietane-type and pimarane-type) were found in the DES extracts of *P. pinaster* wood. Compared to organic solvent (eg. acetone), no fatty acids were found in the DES extracts. Also, hydroxy resin acids were not seen. In between all DES types and all experimental conditions, dehydroabietic acid (18-37 %) and abietic acid (14-28 %) were found to be the major compounds (Table 5 and Table 6). Isopimaric acid, a pimarane type, was determined as 7.3-15 % in both DES. However, the highest amount was obtained with ChCl:Et-Gly in HW at 60 °C from 1 g of *P. pinaster* wood. With the organic solvent (n-hexane), the amount of isopimaric acid was 7.7 %.

PSMME (pinosylvin monomethyl ether) a typical stilbene for Pinus species was not found in ultrason bath extraction with ChCl:Ur (Table 6), whereas in hot water extraction (HW) the amount of PSMME was 0.4-2.4 %. ChCl:Ur is more viscous and it is difficult to handle during silylation.

Table 4 Amount of lipophilics extracted with organic solvents from *P.pinaster* wood (%)
Tablica 4. Udio lipofilnih spojeva dobivenih ekstrakcijom iz drva primorskog bora (*Pinus pinaster*) organskim otapalima (%)

No *Broj*	**RT** *Retencijsko vrijeme*	**Compounds** *Spojevi*	**n-Hexane** *n-heksan*	**Acetone** *Aceton*
1	18.274	16:00	0.4	-
2	20.925	linoleic (18 : 2) / *linolna kiselina (18 : 2)*	0.7	11.4
3	20.994	oleic (18:3) / *oleinska kiselina (18 : 3)*	1.1	38.8
4	21.36	18 : 0	-	6.8
5	22.343	PSMME	0.6	25
6	22.658	pimaric acid / *pimarna kiselina*	8.7	-
7	22.889	sandracopimaric acid / *sandrakopimarna kiselina*	1.4	-
8	23.087	isopimaric acid / *izopimarna kiselina*	7.7	-
9	23.370	palustric acid / *palustrinska kiselina*	10.2	-
10	23.702	levopimaric acid / *levopimarna kiselina*	15.9	-
11	23.810	dehydroabietic acid / *dehidroabietinska kiselina*	17.3	11.5
12	24.245	abietic acid / *abietinska kiselina*	18.2	6.7
13	25.622	neoabietic acid / *neoabietinska kiselina*	11.8	-
14	26.545	hydroxy-resin1 / *hidroksi-smola 1*	1.8	-
15	26.866	hydroxy-resin2 / *hidroksi-smola 2*	2.1	-
16	27.265	hydroxy-resin3 / *hidroksi-smola 3*	2.1	-

Table 5 Amount of lipophilics extracted from *P. pinaster* wood with ChCl:Et-Gly (%)
Tablica 5. Udio lipofilnih spojeva dobivenih ekstrakcijom iz drva primorskog bora (*Pinus pinaster*) smjesom ChCl : Et-Gly (%)

Solid / Liquid ratio *Odnos kruto – tekuće*	**Compounds** *Spojevi*	**UAE**				**HW**			
		40 °C		**60 °C**		**40 °C**		**60 °C**	
		30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min
1 : 10, g/mL	18 : 00	4.0±2.7	3.1±0.3	2.0±0.2	2.4±0.3	3.3±1.0	6.5±0.2	2.5±0.8	5.0±1.2
	PSMME	1.2±0.5	1.2±1.0	3.3±1.0	2.3±2.4	3.8±2.8	1.3±0.3	4.0±1.8	2.1±1.0
	Pimaric acid / *pimarna kiselina*	10±0.8	9.6±0.6	9.2±0.6	9.6±1.0	9.2±1.7	8.5±0.0	9.3±2.1	8.0±0.1
	Pimaric acid / *pimarna kiselina*	1.3±0.1	1.4±0.2	1.3±0.5	1.5±0.1	1.3±0.2	1.0±0.1	1.3±0.1	1.7±0.3
	Isopimaric acid *izopimarna kiselina*	11±0.1	10±0.1	10±0.6	10±0.2	10±1.3	11±0.6	10±1.1	15±1.9
	Palustric acid *palustrinska kiselina*	12±0.7	12±0.4	8.9±1.6	11±2.0	10±2.2	12±1.7	9.7±2.8	13±1.3
	Levopimaric acid *levopimarna kiselina*	9.8±0.5	10±1.7	3.8±2.1	9.7±1.4	5.0±4.1	7.6±0.8	4.2±4.4	3.2±0.4
	Dehydroabietic acid *dehidroabietinska kiselina*	29±0.5	27±2.7	38±0.6	28±3.2	33±2.5	29±0.1	35 ±3.6	31±1.5
	Abietic acid *abietinska kiselina*	17±1.6	18±0.3	18±2.3	19±0.3	18±3.1	19±0.6	18 ±3.0	14±1.1
	Neoabietic acid *neoabietinska kiselina*	5.3±2.3	6.6±1.5	5.1±0.2	6.2±1.0	5.7±0.2	5.3±1.4	4.8 ±1.2	9.6±5.0
1 : 20, g/mL	18 : 00	3.2±0.8	2.6±0.1	3.1±0.4	3.1±0.5	2.8±0.0	5.1±2.2	2.4±0.4	8.0±0.7
	PSMME	4.6±0.3	2.1±0.3	3.8±0.9	1.7±0.4	5.5±1.5	2.9±0.6	3.3±0.3	1.9±0.4
	Pimaric acid / *pimarna kiselina*	11±0.6	12±0.2	10±0.5	12±0.0	8.2±2.0	9.0±2.1	11±1.1	7.9±0.4
	Pimaric acid / *pimarna kiselina*	1.2±0.1	1.4±0.2	1.2±0.1	1.4±0.2	1.2±0.3	1.3±0.0	1.4±0.2	1.4±0.1
	Isopimaric acid *izopimarna kiselina*	9.4±0.6	11±0.0	9.8±1.2	11±0.0	9.6±2.0	9.7±1.2	10±0.2	9.5±0.3
	Palustric acid *palustrinska kiselina*	10±0.5	12±0.3	9.7±0.4	12±0.4	11±2.2	12±0.1	11±0.5	11±0.1
	Levopimaric acid *levopimarna kiselina*	6.4±1.5	9.1±0.6	5.7±0.9	8.6±0.7	5.7±0.8	5.6±2.4	6.4±0.6	6.2±1.3
	Dehydroabietic acid *dehidroabietinska kiselina*	36±3.1	29±1.4	37±2.3	29±0.8	33 ±4.0	30±3.5	34±2.0	28±0.4
	Abietic acid *abietinska kiselina*	14±0.5	16±0.1	16±0.4	17±0.5	17 ±0.4	18±3.2	16±0.4	19±1.0
	Neoabietic acid *neoabietinska kiselina*	4.1±0.1	4.7±0.2	3.9±0.2	4.0±0.3	6.1±1.5	6.0±3.0	3.7±0.3	6.9±0.6

Table 6 Amount of lipophilics extracted from *P.pinaster* wood with ChCl:Ur (%)
Tablica 6. Udio lipofilnih spojeva dobivenih ekstrakcijom iz drva primorskog bora (*Pinus pinaster*) smjesom ChCl : Ur (%)

Solid / Liquid ratio *Odnos kruto – tekuće*	**Compounds** *Spojevi*	**UAE**				**HW**			
		40 °C		**60 °C**		**40 °C**		**60 °C**	
		30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min
1 : 10, g/mL	18 : 00	9.0±2.3	4.1±0.7	9.4±1.5	2.9±0.1	5.5±2.7	4.7±0.4	6±3.4	5.2±0.9
	PSMME	-	-	-	-	0.9±0.1	1.2±0.0	1.6±0.5	2.4±1.0
	Pimaric acid / *pimarna kiselina*	7.0±0.0	7.2±0.0	7.8±0.4	7.7±0.0	7.9±0.5	8.1±0.2	7.7±0.7	6.0±0.6
	Pimaric acid / *pimarna kiselina*	0.9±1.3	0.7±1.0	1.4±0.1	1.4±0.0	1.5±0.1	1.5±0.0	1.4±0.1	1.2±0.0
	Isopimaric acid *izopimarna kiselina*	8.4±0.3	8.3±0.6	8.8±0.1	8.9±0.3	9.5±0.2	9.3±0.5	9.3±0.7	8.8±1.0
	Palustric acid *palustrinska kiselina*	9.9±0.1	9.7±1.7	9.0±0.5	10±0.1	7.2±0.1	8.1±1.2	8.7±1.2	11±0.5
	Levopimaric acid *levopimarna kiselina*	9.5±1.7	12±1.0	10±1.0	11±0.0	±7.9±0.3	7.8±0.6	8.6±1.3	7.2±1.6
	Dehydroabietic acid *dehidroabietinska kiselina*	25±1.5	27±0.8	24±1.0	26±0.9	29±1.9	30±0.2	27±3.0	28±2.6
	Abietic acid *abietinska kiselina*	20±1.1	19±0.5	18±0.3	20±0.1	21±0.3	21±0.5	20±1.4	19±0.5
	Neoabietic acid *neoabietinska kiselina*	9.9±0.5	12±1.6	10±0.3	11±0.1	9.1±0.3	8.7±1.0	9.7±0.0	11±2.6
1 : 20, g/mL	18 :00	9.2±3.0	9.8±1.4	9.0±5.2	7.7±2.3	29±2.9	25±1.3	24±0.2	28±4.1
	PSMME	-	-	-	-	0.4±0.0	0.6±0.2	0.9±0.2	1.0±0.1
	Pimaric acid / *pimarna kiselina*	6.2±0.8	7.9±0.1	7.0±1.3	8.1±0.2	3.6±3.5	6.3±0.3	5.9±0.6	5.1±1.1
	Pimaric acid / *pimarna kiselina*	0.9±0.5	1.4±0.0	1.4±0.0	1.6±0.3	4.9±5.4	1.5±0.2	1.0±0.1	1.0±0.0
	Isopimaric acid izopimarna kiselina	8.6±0.3	9.8±0.5	9.3±0.2	8.8±0.7	7.3±0.1	8.8±0.7	11±1.6	12±0.5
	Palustric acid *palustrinska kiselina*	8.4±1.2	9.5±0.5	9.6±0.8	9.1±0.9	5.9±0.3	7.2±1.2	6.6±0.4	7.1±0.2
	Levopimaric acid *levopimarna kiselina*	8.7±0.1	9.0±3.0	12±0.9	12±0.7	12±6.0	6.3±0.9	6.9±0.9	6.6±0.1
	Dehydroabietic acid *dehidroabietinska kiselina*	23±0.8	24±2.3	21±0.8	22±1.5	19±3.	19±0.6	20±0.6	18±2.1
	Abietic acid *abietinska kiselina*	28±1.7	19±0.0	20±0.4	20±0.6	11±6.0	18±1.5	18±0.9	16±0.5
	Neoabietic acid *neoabietinska kiselina*	7.4±1.3	9.1±0.7	11±0.9	11±0.3	6.9±0.3	7.9±0.5	6.2±0.8	4.8± 2

The effects of experimental conditions, e.g. time and temperature, on the main compounds like dehydroabietic, abietic and isopimaric acids are shown in Figure 1 and 2. As seen, the amount of the main compounds are higher with ChCl:Et-Gly than with ChCl:Ur. At the optimum conditions discussed in Taguchi analysis (solid:liquid ratio of 1:10, extraction temperature: 60 °C, extraction time: 60 min, extraction method HW), dehydroabietic acid was found to be (31.1 ± 1.5) % with ChCl:Et-Gly and (28±3) % with ChCl:Ur. The second important compound, abietic acid, was (16.2±1.1) % and (20±0.5) % in ChCl:Et-Gly and ChCl:Ur, respectively.

Temperature is another factor affecting the efficiency of extraction. Increasing the temperature from 40 to 60 °C, changed the amount of dehydroabietic acid from (28.7±0.1) % to (31.1±1.5) %. For isopimaric acid, this amount is (10.8±0.6) % to (13.1±1.9) % respectively. Increasing the temperature, decreased the solvent viscosity and mass transfer limitations, but increased the diffusivity. Similar results were obtained by Ozturk *et al*. (2018b) for polyphenolics from orange peel.

Solid:liquid ratio is an important parameter for the operation. The increase of the amount of liquid increases positive interaction between the solid and liquid states. Two different ratios were applied in this study (1:10 and 1:20 g/mL). Changing the liquid ratio from 10 mL to 20 mL, decreased the amount of dehydroabietic acid (31.1±1.5 – 28±0.4 %). Similar decline was observed for isopimaric acid (13.1±1.9 – 9.5±0.3 %). However, for abietic acid, increasing the liquid ratio positively affects the amount (16.2±1.1 – 18.7±1.0 %).

As seen in Figure 1, at the optimum conditions, the amount of dehydroabietic was found to be (31.1±1.5) % with hot water bath and (28±3.2) % with UAE. To the contrary, the amount of abietic acid was found low with HW. UAE is a simple method that requires less time and solvent. In the recent studies, UAE

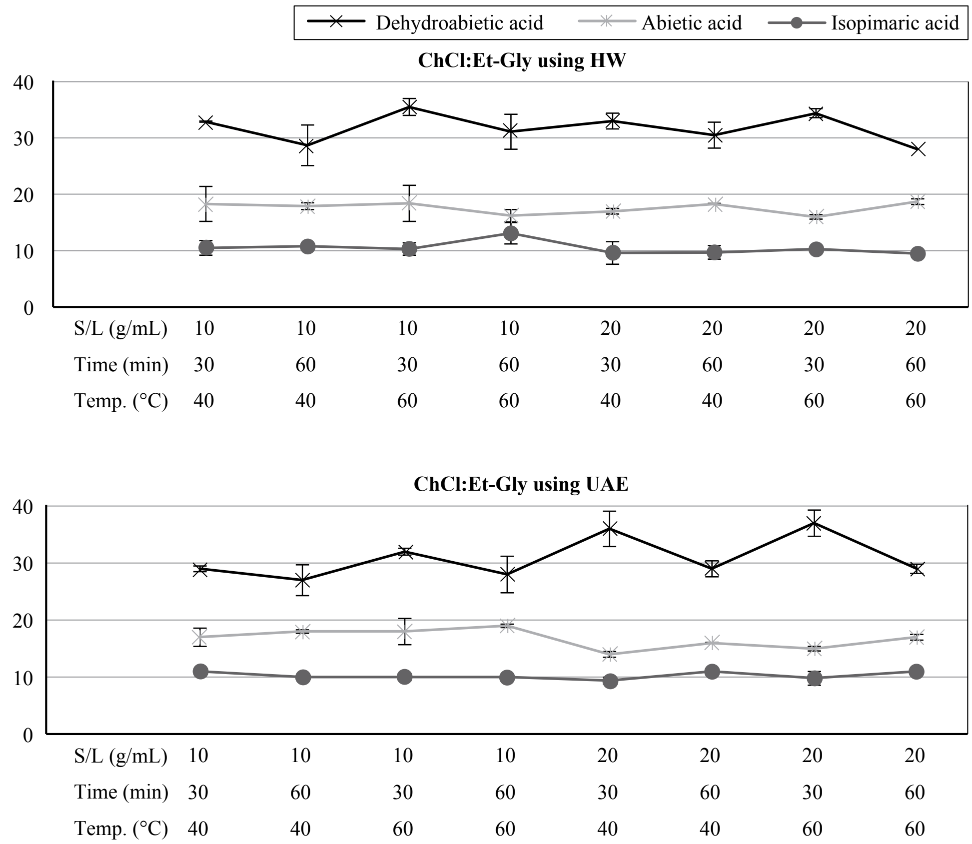

Figure 1 The amount of main compounds extracted with ChCl:Et-Gly using HW and UAE methods
Slika 1. Udjeli osnovnih spojeva ekstrahiranih smjesom ChCl: Et-Gly uz primjenu vruće vode i ultrazvuka

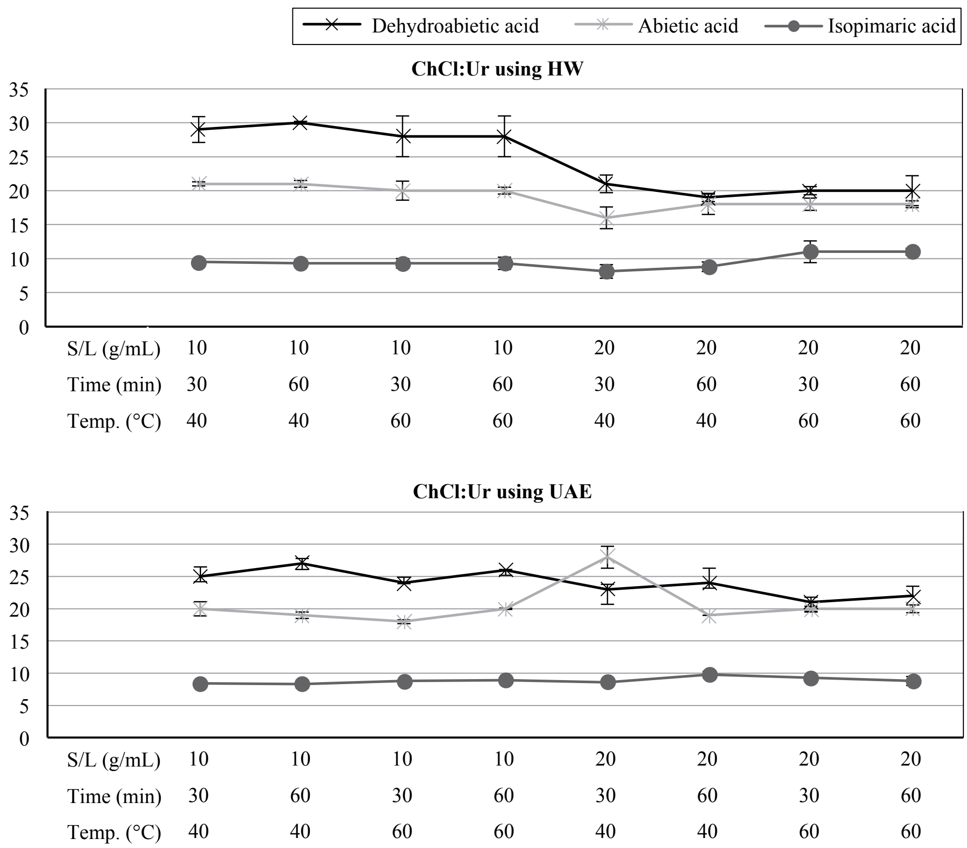

Figure 2 The amount of main compounds extracted with ChCl:Ur using HW and UAE methods
Slika 2. Udjeli osnovnih spojeva ekstrahiranih smjesom ChCl : Ur uz primjenu vruće vode i ultrazvuka

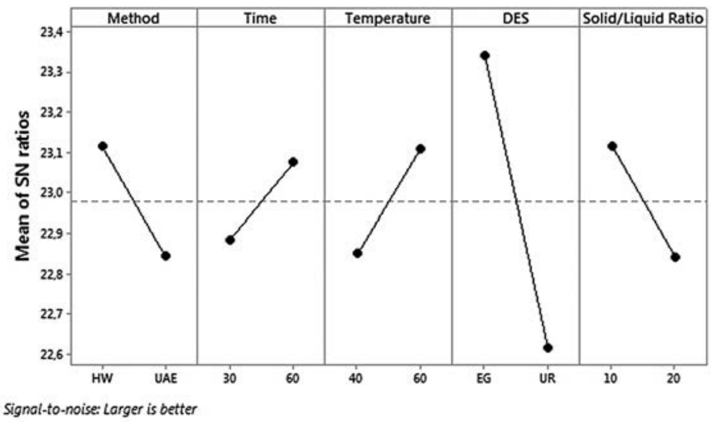

Figure 3 Main effect plots of S/N ratio
Slika 3. Dijagrami glavnog učinka omjera S/N

Table 7 S/N ratios of factor levels for experimental parameter
Tablica 7. Omjeri signala i šuma (S/N) razina faktora za eksperimentalne parametre

Level *Razina*	**A**	**B**	**C**	**D**	**E**
1	23.11	22.88	22.85	23.34	23.12
2	22.84	23.07	23.11	22.62	22.84
Delta	0.27	0.19	0.26	0.73	0.27
Rank *Rang*	3	5	4	1	2

and microwave extraction (MW) have started to be applied to biomass, however, mainly using water or oil baths (Skulcova *et.al*., 2018; Li *et.al*., 2017; Chen and Wan, 2018; Gülsoy and Kilic-Pekgözlü, 2021).

3.2 Taguchi analysis
3.2. Taguchijeva analiza

S/N ratios related to control parameters obtained from Taguchi analysis are given in Table 7. The highest S/N of each parameter indicates the optimal level of that parameter. For example, as seen in Table 7, the 23.11 S/N ratio value in the method factor is defined as level 1 and shows that HW is the method with the best results. Likewise, with the 23.24 S/N ratio value, Et-Gly is the best DES solvent. Overall, among all parameters, the most effective factor was DES solvent type.

As seen in Figure 3. the greatest S/N ratio ensures the best levels of experimental parameters. The optimal factors for this study were determined as A: Hot water bath extraction; B: 60 min.; C: 60 °C; D: Ethylene glycol; E: 1:10 solid:liquid ratio.

Table 8 Values of Grey relational grade
Tablica 8. Vrijednosti ocjena sive relacijske analize

No *Broj*	**A**	**B**	**C**	**D**	**E**	**Grey Grade** *Ocjena prema sivoj relacijskoj analizi*	**Rank** *Rang*	**No** *Broj*	**A**	**B**	**C**	**D**	**E**	**Grey Grade** *Ocjena prema sivoj relacijskoj analizi*	**Rank** *Rang*
1	1	1	1	1	1	0.5321	7	17	2	1	1	1	1	0.4873	11
2	1	1	1	1	2	0.4993	10	18	2	1	1	1	2	0.5455	5
3	1	1	1	2	1	0.4797	16	19	2	1	1	2	1	0.5003	9
4	1	1	1	2	2	0.3539	32	20	2	1	1	2	2	0.5828	4
5	1	1	2	1	1	0.5835	3	21	2	1	2	1	1	0.5004	8
6	1	1	2	1	2	0.5387	6	22	2	1	2	1	2	0.5937	2
7	1	1	2	2	1	0.4545	19	23	2	1	2	2	1	0.3962	30
8	1	1	2	2	2	0.4338	23	24	2	1	2	2	2	0.4078	28
9	1	2	1	1	1	0.4835	14	25	2	2	1	1	1	0.4440	22
10	1	2	1	1	2	0.4745	17	26	2	2	1	1	2	0.4804	15
11	1	2	1	2	1	0.4864	13	27	2	2	1	2	1	0.4179	27
12	1	2	1	2	2	0.3709	31	28	2	2	1	2	2	0.4259	26
13	1	2	2	1	1	0.6588	1	29	2	2	2	1	1	0.4613	18
14	1	2	2	1	2	0.4464	21	30	2	2	2	1	2	0.4873	11
15	1	2	2	2	1	0.4545	19	31	2	2	2	2	1	0.4299	25
16	1	2	2	2	2	0.4338	23	32	2	2	2	2	2	0.4031	29

3.3 Grey relations analysis
3.3. Siva relacijska analiza

Table 8 shows grey relational grade values obtained by using Eqs. 2, 3 and 4. As seen from the table, the optimum parameters were observed in the experiment number 13, which has the highest grey grade value (0.6588). These parameters belong to the combination of experiments, as obtained in the Taguchi analysis. A: Hot water bath extraction; B: 60 min.; C: 60 °C; D: Ethylene glycol; E:1:10 solid:liquid ratio (g/mL).

4 CONCLUSIONS
4. ZAKLJUČAK

Extraction of lipophilic compounds, e.g fatty and resin acids which have antimicrobial and antifungal activities, was investigated with deep eutectic solvents. Choline chloride based on two different eutectic mixture urea (1:2) and ethylene glycol (1:2) was used. Ten different compounds, mainly resin acids, were identified in the DES mixtures with GC-MS. Dehydroabietic acid, abietic acid, isopimaric acid and palustric acid were found to be the major compounds. L_{32} orthogonal array from Taguchi was applied for optimization. Extraction method, extraction time, extraction temperature, solid:liquid ratio and DES type were the main parameters.

The sequence of individual parameters in this study is ranked as follows: DES type > solid:liquid ratio > extraction method > temperature > time. Ethylene glycol was found to be more effective compared to urea to extract the lipophilic compounds from *Pinus pinaster* wood. 1 g of wood meal and 10 mL of DES mixture (solid:liquid ratio) were found to be sufficient. Hot water extraction at 60 °C for 60 min. are the optimum factors for this study. Compared to traditional wood extraction with soxhlet apparatuses, DES application needs only 1 g of wood meal and 60 min.

Further, with different DES mixtures and wood species, more effective extraction methods can be developed in the concept of green chemistry and wood extractives.

Acknowledgements – Zahvala

This study was supported by Bartin University of Scientific Research Project Unit (BAP-2018-FEN-A-006). The co-author, Rıfat KURT, performed the Taguchi analysis, which was not done in this project.

5 REFERENCES
5. LITERATURA

1. Aydın, I., 2017: Resin production and turpentine analysis by acid paste and borehole methods in red pine and maritime pine in Turkey. Master Thesis, Karadeniz Technical University, Graduate School of Natural and Applied Sciences.
2. Barbieria, J.; Goltz, C.; Cavalheiro, F. B.; Toci, A. T.; Igarashi-Mafra, L.; Mafra, M. R., 2020: Deepeutectic solvents applied in the extraction and stabilization of rosemary (*Rosmarinus officinalis* L.) phenolic compounds. Industrial Crops and Products, 144: 112049. https://doi.org/10.1016/j.indcrop.2019.112049
3. Benouadah, N.; Pranovich, A.; Aliouche, D.; Hemming, J.; Smends, A.; Willför, S., 2018: Analysis of extractives from *Pinus halepensis* an *Eucalyptus camaldulensis* as predominant trees in Algeria. Holzforschung, 72 (2): 97-104. https://doi.org/10.1515/hf-2017-0098
4. Bubalo, M. C.; Vidović, S.; Radojčić Redovniković, I.; Jokić, S., 2015: Green solvents for green technologies. Journal of Chemical Technology and Biotechnology, 90: 1631-1639. http://dx.doi.org/10.1002/jctb.4668
5. Cao, J.; Wang, H.; Zhang, W.; Cao, F.; Ma, G.; Su, E., 2018: Tailor-made deep eutectic solvents for simultaneous extraction of five aromatic acids from ginkgo biloba leaves'. Molecules, 23 (12): 3214. https://doi.org/10.3390/molecules23123214
6. Chen, D.-C.; Lin, J.-Y.; Jheng, M.-W.; Chen, J.-M., 2007: Design of titanium alloy superplastic blow-forming in ellip-cylindrical die using Taguchi method. In: Proceedings of the 35th International MATADOR Conference. Springer, London, pp. 105-109.
7. Chen, Z.; Wan, C., 2018: Ultrafast fractionation of lignocellulosic biomass by microwaveassisted deep eutectic solvent pretreatment. Bioresource Technology, 250: 532-537. https://doi.org/10.1016/j.biortech.2017.11.066
8. Conde, E.; Fang, W.; Hemming, J.; Willför, S.; Domiguez, H.; Parajo, J. C., 2014: Recovery of bioactive compounds from *Pinus pinaster* wood by consecutive extractive stages. Wood Science and Technology, 48: 311-323. https://doi.org/10.1007/s00226-013-0604-1
9. Fengel, D.; Wegener, G., 2003: Wood: Chemistry, ultrastructure, reaction, Verlag Kessel.
10. Fischer, V., 2015: Properties and applications of deep eutectic solvents and low-melting mixtures. PhD Thesis, Naturwissenschaften an der Fakultät für Chemie und Pharmazie der Universität Regensburg.
11. Gunay, M.; Yucel, E., 2013: Application of Taguchi method for determining optimum surface roughness in turning of high-alloy white cast iron. Measurement, 46 (2): 913-919. https://doi.org/10.1016/j.measurement.2012.10.013
12. Gunay, M.; Kacal, A.; Turgut, Y., 2011: Optimization of machining parameters in milling of Ti-6Al-4V alloy using Taguchi method. e-Journal of New World Sciences Academy, 6 (1): 428-440. https://doi.org/10.12739/NWSAES.V6I1.5000067046
13. Gülsoy, S. K.; Kilic-Pekgözlü, A., 2021: Derin Ötektik Çözücüler ve Delignifikasyon Uygulamaları. Ziraat, Orman ve Su Ürünlerinde Araştırma ve Değerlendirmeler, Gece Kitaplığı, 10 (2).
14. Häkkinen, R., 2020: Carbohydrates in Deep Eutectic Solvents. PhD Thesis, Faculty of Science, University of Helsinki, Finland.
15. Haq, A. N.; Marimuthu, P.; Jeyapaul, R., 2008: Multi response optimization of machining parameters of drilling Al/SiC metal matrix composite using grey relational analysis in the Taguchi method. The International Journal of Advanced Manufacturing Technology, 37: 250-255. https://doi.org/10.1007/s00170-007-0981-4

16. Jiang, L.; Li, Y.; Huang, Y.; Yu, J.; Qiao, X.; Wang, Y.; Huang, C.; Cao, Y., 2020: Optimization of multi-stage constant current charging pattern based on Taguchi method for Li-Ion battery. Applied Energy, 259: 114148. https://doi.org/10.1016/j.apenergy.2019.114148
17. Koch, P., 1972: Utilization of Southern Pine, Vol.1. Agriculture Handbook No:420.USA.
18. Kumar, R. S.; Sureshkumar, K.; Velraj, R., 2015: Optimization of biodiesel production from Manilkara zapota (L.) seed oil using Taguchi method. Fuel, 140: 90-96.
19. Kurtca, M.; Tumen, I., 2020: Investigation into seasonal variations of chemical compounds of maritime pine grown in Turkey. Fresenius Environmental Bulletin, 29 (09A): 8156-8167.
20. Kurt, M.; Bagci, E.; Kaynak, Y., 2009: Application of Taguchi methods in the optimization of cutting parameters for surface finish and hole diameter accuracy in dry drilling progress. International The international Journal of Advanced Manufacturing Technology, 40: 458-469. https://doi.org/10.1007/s00170-007-1368-2
21. Kurt, R.; Can, A., 2021: Optimization of the effect of accelerated weathering conditions on wood surfaces via the Taguchi method. BioResources, 16 (1): 1642-1653. https://doi.org/10.15376/biores.16.1.1642-1653
22. Liu, Y.; Liu, C.; Liu, W.; Ma, Y.; Tang, S.; Liang, C.; Cai, Q.; Zhang, C., 2019: Optimization of parameters in laser powder deposition AlSi10Mg alloy using Taguchi method. Optics and Laser Technology, 111: 470-480. https://doi.org/10.1016/j.optlastec.2018.10.030
23. Li, T.; Lyu, G.; Liu, Y.; Lou, R.; Lucia, L. A.; Yang, G.; Chen, J.; Saeed, H. A., 2017: Deep eutectic solvents (DESs) for the Isolation of Willow Lignin (*Salix matsudana* cv. Zhuliu). International Journal of Molecular Sciences, 18: 2266. https://doi.org/10.3390/ijms18112266
24. Meng, Z.; Zhao, J.; Duan, H.; Guan, Y.; Zhao, L., 2018: Green and efficient extraction of four bioactive flavonoids from Pollen Typhae by ultrasound-assisted deep eutectic solvents extraction. Journal of Pharmaceutical and Biomedical Analysis, 161: 246-253. https://doi.org/10.1016/j.jpba.2018.08.048
25. Ozakin, A. N.; Kaya, F., 2020: Experimental thermodynamic analysis of air-based PVT system using fins in different materials: Optimization of control parameters by Taguchi method and ANOVA. Solar Energy, 197: 199-211. https://doi.org/10.1016/j.solener.2019.12.077
26. Ozturk, B.; Esteban, J.; Gonzales-Miquel, M., 2018a: Determination of citrus essential oils using glycerol-based deep eutectic solvents. Journal of Chemical and Engineering, 63 (7): 2384-2393. https://doi.org/10.1021/acs.jced.7b00944
27. Ozturk, B.; Parkinson, C.; Gonzales-Miquel, M., 2018b: Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents. Separation and Purification Technology, 206: 1-13. https://doi.org/10.1016/j.seppur.2018.05.052
28. Panda, A.: Sahoo, A.; Rout, R., 2016: Multi-attribute decision making parametric optimization and modeling in hard turning using ceramic insert through grey relational analysis: A case study. Decision Science Letters, 5 (4): 581-592. https://doi.org/10.5267/j.dsl.2016.3.001
29. Shi, K.; Zhang, D.; Ren, J., 2015: Optimization of process parameters for surface roughness and microhardness in dry milling of magnesium alloy using Taguchi with grey relational analysis. The International Journal of Advanced Manufacturing Technology, 81 (1): 645-651. https://doi.org/10.1007/s00170-015-7218-8
30. Sjöström, E., 1981: Wood Chemistry:Fundamentals and Application. Academic Press, USA.
31. Skulcova, A.; Russ, A.; Jablonsky, M.; Sima, J., 2018: The pH behavior of seventeen deep eutectic solvents. BioResources, 13: 5042-5051.
32. Sousa, J. L; Ramos, P. A. B.; Freire, C. S. R; Silva, A. M. S.; Sivestre, A. J. D., 2018: Chemical composition of lipophilic bark extracts from *Pinus pinaster* and *Pinus pinea* cultivated in Portugal. Applied Sciences, 8: 2575. https://doi.org/10.3390/app8122575
33. Sun, J.; Yang, Y.; Wang, D., 2013: Parametric optimization of selective laser melting for forming Ti6Al4V samples by Taguchi method. Optics and Laser Technology, 49: 118-124.
https://doi.org/10.1016/j.optlastec.2012.12.002
34. Taguchi, G.; Chowdhury, S.; Wu, Y., 2005: Taguchi's Quality Engineering Handbook. John Wiley & Sons, Inc., Hoboken, NJ.
35. Tosun, N., 2005: Determination of optimum parameters for multi-performance characteristics in drilling by using grey relational analysis. The International Journal of Advanced Manufacturing Technology, 28 (5): 450-455. https://doi.org/10.1007/s00170-004-2386-y
36. Uslu, S.; Aydın, M., 2020: Effect of operating parameters on performance and emissions of a diesel engine fueled with ternary blends of palm oil biodiesel/diethyl ether/diesel by Taguchi method. Fuel, 275: 117978.
https://doi.org/10.1016/j.fuel.2020.117978
37. Vek, V.; Poljanšek, I.; Humar, M.; Willför, S.; Oven, P., 2020: In vitro inhibition of extractives from knotwood of Scots pine (*Pinus sylvestris*) and black pine (*Pinus nigra*) on growth of *Schizophyllum commune*, *Trametes versicolor*, *Gloeophyllum trabeum* and *Fibroporia vaillantii*. Wood Science and Technology, 54: 1645-1662. https://doi.org/10.1007/s00226-020-01229-7
38. Velioğlu, E.; Bostancı, Y. S.; Akgül, S., 2020: Poplars, Willows and other-fast growing trees in Turkey: Country Progress Report for the International Poplar Commission Time period: 2016-2019.
39. Yang, C. C.; Chen, B. S., 2006: Supplier selection using combined analytical hierarchy process and grey relational analysis. Journal of Manufacturing Technology Management, 17 (7): 926-941.
https://doi.org/10.1108/17410380610688241
40. Zdanowicz, M.; Wilpiszewska, K.; Spychaj, T., 2018: Deep eutectic solvents for polyssharides processing. A review. Carbonhydrate Polymers, 200: 361-380.
https://doi.org/10.1016/j.carbpol.2018.07.078

Corresponding address:

Prof. Dr. Ayben KILIC-PEKGÖZLÜ
Bartin University, Forestry Faculty, 74100, Bartin, TURKEY, e-mail: akilic@bartin.edu.tr