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1 **Green and Innovative Extraction of Polyphenols from**
2 ***Rhamnus alaternus* using Natural Deep Eutectic Solvents**
3 **and Evaluation of their Bioactivities**

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24

25 **Abstract**

26 Phenolic compounds, naturally present in plants, are particularly relevant as nutraceuticals for
27 their antioxidant activities. Here, choline chloride-based natural deep eutectic solvents
28 (NaDESs) were used for efficient extraction of total phenolic compounds (TPC) from leaves,
29 pods and roots of *Rhamnus alaternus* plant. In each extract, the polyphenols content and their
30 antioxidant activities were evaluated. More, their antibacterial activity was carried out against
31 Gram-positive and Gram-negative bacteria. The investigated extracts presented antioxidant and
32 antimicrobial activities. The leaves, pods and roots extracts obtained using the choline chloride-
33 glycerol (ChCl-Gly) mixture exhibited a high value of TPC comprised within 437.7 and 317.0 mg
34 GAE/100 g d.w. and showed the best antioxidant properties with ABTS radical scavenging
35 capacity (IC₅₀: from 44.55 to 53.56 µg/mL), DPPH assay (IC₅₀: from 26.36 to 118.23 µg/mL),
36 reducing activity (EC₅₀: from 77.53 to 85.05 µg/mL) and iron chelation activity (EC₅₀: from 91.73
37 to 108.7 µg/mL). In contrast, choline chloride-urea (ChCl-Ur) extracts showed a low antioxidant
38 capacity. Lastly, the best bioactive extracts were characterized using HPLC-MS/MS to determine
39 their bioactive compounds. The present study suggests that the DES-based method developed
40 was selective, efficient and sustainable for extraction of polyphenols.

41

42 **Keywords:** *Rhamnus alaternus*, natural deep eutectic solvents, green extraction, sustainable
43 process, bioactive compounds, antioxidant, antimicrobial activity.

44

45

46 1. Introduction

47 Recently, the green extraction techniques have become of utmost importance in research
48 related to biomolecules production. Therefore, food, cosmetic and pharmaceutical industries
49 operated a transition from conventional extraction to the green extraction processes, using
50 alternative cost-effective and earth-friendly solvents (Nagarajan et al., 2019; Palos-Hernández
51 et al., 2022). Considering environmental protection and collective awareness, consumers are
52 more and more demanding for highly valued bio-based products (Bugge et al., 2016; Catone et
53 al., 2021). Plants are an important source of various bioactive molecules notably polyphenolic
54 compounds (Si et al., 2016), known for their pharmacological properties including antioxidant,
55 antibacterial, anti-inflammatory and anticancer effects (Zhang et al., 2011).

56 *Rhamnus alaternus*, popularly called “Imlilisse”, belongs to the *Rhamnaceae* family and the
57 *Reynosia* Genus. Widely distributed around the Mediterranean region, this plant has been
58 widely consumed as infusion for thousands of years, notably for treating dermatological
59 complications and for its hypotensive, laxative, purgative effect (Ben Ammar et al., 2007).
60 Recent investigations have demonstrated the health benefits and high functional activities of
61 bioactive compounds present in *R. alaternus* including antioxidant, antibacterial,
62 antihyperlipidemic, antigenotoxic and antimutagenic activities (Bhourri et al., 2011; Tacherfiout
63 et al., 2018). The main bioactive substances identified in various parts of *R. alaternus* are
64 polyphenols, flavonoids, anthraquinones, coumarin and anthocyanins (Longo et al., 2005;
65 Moussi et al., 2015). Polyphenols are the most active constituents in secondary metabolites of
66 plants with high biological activities including antioxidant capacity (Quideau et al., 2011).

67 To date, the extraction of bioactive compounds from *R. alaternus* is largely carried out using
68 conventional solid-liquid extraction methods involving organic solvents such as ethanol,
69 acetone and ethyl acetate (Boussahel et al., 2015). However, the consumption of such solvents
70 may contribute to environmental pollution due to their toxicity, volatility and flammability. In
71 this regard, at the beginning of the 2000's, Natural Deep Eutectic Solvents (**NaDESs**) emerged as
72 alternative for green chemistry. NaDESs were simply prepared by mixing hydrogen bond donors
73 (**HBD**) and hydrogen bond acceptors (**HBA**) (Trivedi et al., 2016). Characterized by their low
74 toxicity, biodegradability, high solubility, biocompatibility, low vapor pressure and being eco-
75 friendly, NaDESs are appropriate for substituting conventional solvents (Jeong et al., 2015).
76 Furthermore, NaDESs were successfully applied for the extraction of bioactive compounds such
77 as polyphenols, flavonoids and saponins from natural resources (Dai et al., 2013; Sillero et al.,
78 2021).

79 To the author's knowledge, the extraction of bioactive compounds from *R. alaternus* has never
80 been explored using Natural Deep Eutectic Solvents. Therefore, this study aimed to evaluate
81 the efficiency of three NaDESs, in order to develop green and sustainable process for the
82 extraction of high-value phenolic compounds, from various parts of *R. alaternus* (*i.e.*, leaves,
83 pods and roots) and to investigate the bioactive properties of collected extracts. To reach this
84 aim, three NaDESs were investigated and constituted of choline chloride as HBA and of glycerol,
85 ethylene glycol or urea, as HBD. Compared to conventional ethanol extraction, each extract was
86 evaluated for its total polyphenol content (TPC) and its related bioactivities, notably its
87 antioxidant and antimicrobial activities. Finally, the most promising NaDES extract in terms of
88 TPC was characterized with LC-MS/MS analysis in order to identify its major constituents.

89 2. Material and methods

90 2.1. Plant materials, reagents, equipment and consumable

91 *R. alaternus* was collected from Beni Ourthilane region - Algeria (36°19'57"N,5°5'19"E) in July
92 2019. A voucher specimen (Ra-19-001) was kept in the laboratory of pharmacognosy, pharmacy
93 department, University Salah Boubnider - Constantine 3, Algeria. Dried at room temperature
94 under shadow, the leaves, pods and roots of *R. alaternus* were ground using a cutting mill
95 (SM100 RETSCH, Retsch, Haan, Germany) to obtain a fine powder, stored in controlled
96 atmosphere until use.

97 ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (2,2-diphenyl-1-
98 picrylhydrazyl) and Trolox[®] were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium
99 phosphate dibasic dihydrate and sodium phosphate monobasic dihydrate were provided from
100 VWR Chemicals (Leuven, Belgium). The ascorbic acid, potassium persulfate, ferrozine (3-(2-
101 pycril)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt), iron (II) chloride, EDTA,
102 Folin-Ciocalteu's phenol reagent (2N), gallic acid, potassium ferricyanide (III), iron (III) chloride,
103 iron (III) ferrocyanide, trichloroacetic acid, sodium carbonate, ethylene glycol, choline chloride,
104 urea and glycerol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and
105 ethanol (Sigma-Aldrich) were of analytical grade. Water was purified by Arum[®] 611 water
106 purification system (Sartorius, Göttingen, Germany). For the homogeneity of the unities, all
107 concentrations in the manuscript were presented in mass/volume. CAS number and purity of
108 each chemical used are given in the [Supplementary data \(Table S1\)](#). For antioxidant tests, all
109 spectrophotometry experiments were carried out using Thermo Scientific MultiskanTM
110 spectrophotometer and 96 wells-microplates (Thermo ScientificTM, Roskild, Denmark).

111 **2.2. Solid-liquid extraction**

112 **2.2.1. Natural deep eutectic solvents preparation**

113 Three natural deep eutectic solvents (NaDESs) were selected for the extraction of phenolic
114 compounds from *R. alaternus*'s leaves, pods and roots: choline chloride-glycerol (**ChCl-Gly**),
115 choline-chloride-ethylene glycol (**ChCl-EG**) and choline chloride-urea (**ChCl-Ur**). These NaDESs
116 were prepared as previously described ([Tang & Row, 2020](#); [Wan Mahmood et al., 2019](#)). Briefly,
117 the hydrogen bond acceptor (**HBA**) *i.e.*, choline chloride (**ChCl**) was mixed either with the
118 hydrogen bond donor (**HBD**) *i.e.*, ethylene glycol (**EG**), glycerol (**Gly**) or with urea (**Ur**) at a molar
119 ratio 1:2. These solvents were prepared at 80 °C under stirring using a magnetic agitator (2mag
120 MIX 15; Merck, Darmstadt, Germany) until the formation of a transparent and homogenous
121 liquid (3 - 4 h).

122 The prepared NaDESs were mixed with water (8:2 v/v) to reduce their viscosity and change
123 their polarity in order to increase the extraction efficiency by improving the solubility of TPC in
124 the corresponding solvents ([Chanioti & Tzia, 2018](#)). Indeed, the high viscosity of NaDESs used
125 for extraction process can reduce the mass transport efficiency ([Dai et al., 2013](#); [Wei et al.,](#)
126 [2015](#)). Yet, the water content mixed to NaDESs must not be too high since it could break the
127 hydrogen bonds within the NaDESs constituents ([Vilková et al., 2020](#)).

128 **2.2.2. Extraction process**

129 The phenolic compounds from various part of *R. alaternus* (*i.e.*, leaves, pods and roots) were
130 separated by solid-liquid extraction (**SLE**). Briefly, 1 g of powder obtained from each part of the
131 plant was dissolved in 10 mL of solvent, constituted of one NaDES mixed with water in a 8:2

132 (v/v) ratio. Each SLE experiment was led for 24 h at 30 °C using a thermostated waterbath, and
133 under stirring using a multipoint magnetic stirrer (2mag MIX 15, Merck, Darmstadt, Germany)
134 set at 350 rpm. After extraction, each sample was centrifuged (Hettich universal 320, Sérézin du
135 Rhône, France; 1008 g, 10 min) to remove any solid impurities. The collected supernatants were
136 then filtered through 0.45 µm filter (Millex[®] Syringe Filter, PTFE, Merck KGaA, Darmstadt,
137 Germany) in order to eliminate any remaining particles before further analysis. Each extract
138 was named by its part of the plant (leaves, pods or roots) followed by the nature of the solvent
139 used for SLE extraction (ChCl-Gly, ChCl-EG, ChCl-Ur, or Ethanol). After extraction, each extract
140 was diluted by successive tenfold dilution in ultrapure water until the final dilution factor of
141 1:1000 (v/v) and was used as initial extract for further analysis (polyphenols identification,
142 antioxidant and antimicrobial activity, and HPLC/MS/MS analysis).

143 **2.3. Determination of the total phenolic content**

144 For all investigated extracts, the total phenolic content (**TPC**) was determined by Folin-Ciocalteu
145 method adapted to a microplate assay ([Singleton & Rossi, 1965](#)). Then, 220 µL of ultrapure
146 water, 5 µL of each diluted extract, 15 µL of Folin-Ciocalteu reagent (10 % v/v) and 60 µL of
147 aqueous sodium carbonate (7.5 % w/v) were mixed in each well. Finally, the microplate was
148 stored in the dark for 60 min at ambient temperature, and analysed at 765 nm by
149 spectrophotometry. The absorbance of each sample was measured five times.

150 The total phenolic compound was determined using a calibration curve performed with gallic
151 acid as standard, in a concentration range varying from 0 to 35 µg/mL ($R^2 = 0.998$). The results
152 were expressed as mg gallic acid equivalent (mg **GAE**) per 100 grams of plant powder (d.w.).

153

154 2.4. Determination of antioxidant capacity of the extracts

155 2.4.1. Radical scavenging activity

156 **ABTS radical scavenging activity.** To evaluate the ability of various extracts from *R. alaternus* to
157 scavenge the ABTS^{•+} radical, the method described by [Re et al. \(1999\)](#) was followed. Initially,
158 16.55 mg potassium persulfate was added to 25 mL of the ABTS solution (7 mM) prepared in
159 ultrapure water, allowing the mixture to stand for 12 to 16 h in the dark, at ambient
160 temperature. The concentrated ABTS^{•+} solution was then diluted with 4 mM phosphate buffer
161 pH 7.4 to reach an absorbance value of 0.7 ± 0.02 at 734 nm. Then, 150 μ L of ABTS^{•+} diluted
162 solution was added to 150 μ L of extracts, previously prepared at various concentrations (0 - 120
163 μ g/mL) in the phosphate buffer. After 10 min of incubation, the absorbance was measured at
164 734 nm. Each concentration was repeated five times. The Trolox[®] was investigated as reference
165 from 0 to 25 μ g/mL in the 4 mM phosphate buffer. From the absorbance measurement, the
166 radical scavenging activity (%) was calculated as follows (**Equation 1**) in order to plot a
167 calibration curve (Radical scavenging activity (%) vs. Trolox[®] concentration):

$$168 \text{ Radical scavenging activity (\%)} = \frac{A_{734(\text{control})} - A_{734(\text{sample})}}{A_{734(\text{control})}} \times 100 \quad (1)$$

169 Where $A_{734(\text{control})}$ is the absorbance of the control (initial ABTS^{•+} radical solution; the sample volume
170 was replaced by ultrapure water), and $A_{734(\text{sample})}$ is the absorbance of the remaining radical in the
171 presence of biological extracts or Trolox[®].

171 **DPPH radical scavenging activity.** The ability of various extracts from *R. alaternus* to scavenge
172 the 2,2-diphenyl-1-picrylhydrazyl free radical was evaluated by the DPPH *in vitro* assay similarly
173 to [Brand-Williams et al. \(1995\)](#), with slight modifications. After extraction, each extract was first
174 diluted in ultrapure water. Briefly, 150 μ L of each diluted extract after solid-liquid extraction

175 (final investigated concentration: 0 to 100 µg/mL) was added to 150 µL of the methanolic DPPH°
176 solution (40 µg/mL) daily prepared. Each investigated concentration was repeated 5 times. The
177 mixture was homogenized vigorously using a micropipette for 1 min and stored in the dark at
178 ambient temperature for 30 min. Then, the sample absorbance was read at 517 nm by
179 spectrophotometry. Ascorbic acid (150 µL) was investigated from 5 to 100 µg/mL as standard
180 for plotting the calibration curve. The radical scavenging activity (%) of each extract or standard
181 solution was calculated as follows (**Equation 2**):

$$182 \text{ Radical scavenging activity (\%)} = \frac{A_{517}(\text{control}) - A_{517}(\text{sample})}{A_{517}(\text{control})} \times 100 \quad (2)$$

183 Where $A_{517}(\text{control})$ was the absorbance of the blank DPPH° reagent in the presence of methanol and
184 $A_{517}(\text{sample})$ the absorbance of the DPPH° reagent solution mixed either with the ascorbic acid or the
185 plant extracts.

186 The Inhibitory Concentration 50 (**IC₅₀**) – the concentration that scavenges 50 % of DPPH° free
187 radicals (µg/mL) - was calculated for all *R. alaternus* extracts and compared with the one of
188 ascorbic acid.

189 **2.4.2. Reducing power**

190 The reducing capacity of each biological extract was investigated by the method described by
191 [Oyaizu. \(1986\)](#), with slight modifications. Briefly, 70 µL of various samples extracted and diluted
192 in microplate from (0 to 250 µg/mL) were prepared in phosphate buffer (0.2 M, pH 6.6) and
193 then added with 35 µL of potassium ferricyanide (K₃FeCN₆; 1 % w/v). After 20 min of
194 incubation at 50 °C, 135 µL of ultrapure water was added in each well, followed by 33 µL of
195 trichloroacetic acid (10 % w/v) and 27 µL of iron (III) chloride (FeCl₃; 0.1 % w/v). After 10 min of
196 incubation at 25 °C, the absorbance was measured at 700 nm. Ascorbic acid (70 µL) was

197 investigated as positive control from 5 to 70 µg/mL. Each concentration was repeated 5 times.
198 The reducing capacity of the various extracts was evaluated as follows:

$$199 \text{ Reducing capacity (\%)} = 100 - \left(\frac{A_{700}(\text{control}) - A_{700}(\text{sample})}{A_{700}(\text{control})} \times 100 \right) \quad (3)$$

200 Where: $A_{700}(\text{control})$ is the absorbance of a 66 µM-Prussian blue solution in the absence of ascorbic
201 acid, which was experimentally determined at 0.83 absorbance value at 700 nm,
202 And $A_{700}(\text{sample})$ is the sample or ascorbic acid absorbance at 700 nm.

203 **2.4.3. Iron (II) chelating activity**

204 The ability of various extracts from *R. alaternus* to chelate Fe^{2+} was evaluated using the method
205 described by [Canabady-Rochelle et al. \(2015\)](#) adapted from [Decker & Welch. \(1990\)](#). Briefly, 7.5
206 µL of ferrous chloride solution (FeCl_2 , 253.4 µg/mL) was mixed with 277.5 µL of extract diluted
207 in order to investigate various calculated concentrations (from 0 to 300 µg/mL) in ultrapure
208 water. After 3 min of incubation at 25 °C, 15 µL of ferrozine solution (5 mM) was added in each
209 well. The microplate was shaken using the spectrophotometer agitator, and then incubated for
210 10 min at 25 °C before absorbance measurement at 562 nm. EDTA was investigated as
211 reference from 0 to 10 µg/mL. Each sample and standard were repeated (n = 5). The iron-
212 chelating capacity (%) was calculated as follows (**Equation 4**):

$$\text{Iron - chelation capacity (\%)} = \frac{A_{562}(\text{control}) - A_{562}(\text{sample})}{A_{562}(\text{control})} \times 100 \quad (4)$$

213 Where the blank $A_{562}(\text{control})$ and $A_{562}(\text{sample})$ correspond to the absorbance of ultrapure water in
214 the absence of sample and to the absorbance of sample, both mixed with all reagents.

215

216 **2.5. Antimicrobial activity of the plant extracts**

217 **2.5.1. Antimicrobial test**

218 To evaluate the antimicrobial activity of the samples and NaDESSs, extracts of *R. alaternus* and
219 solvents alone were tested against two Gram+ bacteria (*i.e.*, *Staphylococcus aureus* ATCC 29213
220 (**Sa**) and *Enterococcus faecalis* ATCC 29212 (**Ef**)) and three Gram- bacteria strains (*i.e.*,
221 *Pseudomonas aeruginosa* ATCC 27853 (**Pa**), *Escherichia coli* ATCC 25922 (**Ec**) and *Klebsiella*
222 *pneumoniae* ATCC 700603 (**Kp**)). All strains were obtained from the L2CM laboratory (UMR 7053
223 CNRS-UL, France). The minimum inhibitory concentrations (**MIC**) were determined (**Section**
224 **2.5.3.**).

225 **2.5.2. Preparation of the inoculum**

226 Bacteria cells were grown 18 ± 2 h at 35 °C in Mueller-Hinton broth, cation-adjusted (**MHB-CA**).
227 They were then centrifugated (3000 g, 5 min) and collected pellets were diluted in 10 mL NaCl
228 0.85 % (w/v). The absorbance was recorded at 540 nm with a microplate reader (Thermo
229 Multiskan FC). Bacteria cells were diluted in MHB-CA to 0.5 McFarland turbidity and further
230 diluted to 1:100 to reach a final concentration of $1 [0.4 - 1.6].10^6$ CFU/mL.

231 **2.5.3. MIC determination**

232 The MIC- defined as the lowest concentration of extract with no visible bacterial growth – was
233 determined using the microdilution broth method in 96-well microplates (Eloff, 1998) following
234 the standard (ISO 20776-1, 2019) guidelines with some adaptations.

235 First, solvents (Ethanol and NaDESSs) or extracts were diluted in MHB-CA (final volume: 50 μ L).
236 Then, 50 μ L of bacterial inoculum were added to each well for a total volume of 100 μ L per

237 microplate well (final bacterial concentration: $5 [2 - 8].10^5$ CFU/mL). Growth controls (*i.e.*,
238 bacteria + MHB-CA), sterility control (medium without bacteria), and product control (medium
239 without bacteria with solvents (*i.e.*, Ethanol, ChCl-Gly, ChCl-EG, ChCl-Ur) or extracts) were
240 performed. Each test was repeated 8 times on the microplate. Plates were then incubated $18 \pm$
241 2 h at 35 °C. The MIC was expressed here as the % of extract (v/v) in the wells.

242 First, solvents were investigated alone at 25, 20, 15, 10, 5, 2.5, 1.25, 0.6 and 0.3 % to determine
243 the maximal concentration to use without affecting the cell growth. Then, the extracts were
244 studied from 1 % to 0.004 % (two-fold serial dilution) in a first set of experiments (n = 8). Last,
245 the remaining extracts were investigated at 10, 5, 1, 0.5 and 0.25 % (n = 2 only) in a second
246 series of experiments.

247 **2.6. Characterization of ChCl-Gly extracts by HPLC-MS/MS**

248 The *R. alaternus* leaves, pods and roots extracts using ChCl-Gly were analyzed by mass
249 spectrometry (Bruker Daltonics – micrOTOF – Q LCMS/MS, Bremen, Germany) system. The
250 separation was performed using Hypersil gold C18 column (100 mm × 2.1 mm, 3 µm) at 40 °C.
251 The mobile phase was composed of water containing 0.1% formic acid (solvent A) and
252 acetonitrile 100 % (solvent B). The elution program was set as follows: 95 % A/ 5 % B between 0
253 and 5 min, 1% A/ 99 % B between 5 and 40 min, stay at 99 % B between 40 and 45 min and 95
254 % A/ 5 % B between 45 and 55 min to reach the baseline. Before injection, all extracts were
255 filtered through 0.45 µm membrane filter (J.T. Baker®, Strasbourg, France). Then, 2 µL of each
256 one were injected at 200 µL/min flow rate during the whole experiment.

257 The MS analysis was performed with negative and positive ion modes, scanned m/z from 100 to
258 1000 for the determination of phenolic compounds. Each data was calibrated with Sodium
259 formate at 1 mM in water/isopropanol 1/1. The ESI source conditions were as follows: drying
260 gas at 5 L/min, the nebulizer pressure at 4 bar, the capillary voltage at 4500 V, and the capillary
261 temperature was set at 190 °C. Data acquisitions were executed in autoMSMS mode at 20 eV
262 collision energy.

263 **2.7. Statistical analysis**

264 Data were collected and expressed as the mean \pm standard deviation of three independent
265 experiments and analysed for statistical significance from control, using the Dunnett test SPSS
266 11.5 Statistics Software (SPSS, Chicago, IL, USA). The criterion for significance was set at $p <$
267 0.05. The IC_{50} values, from the in vitro data, were calculated by regression analysis.

268 **3. Results and discussion**

269 **3.1. Determination of total phenolic content**

270 In this study, three different NaDESs (*i.e.*, ChCl-Gly, ChCl-EG, and ChCl-Ur) were investigated for
271 the extraction of TPC from leaves, pods and roots of *R. alaternus* plant and compared to
272 ethanol as conventional solvent and used as reference (Fig. 1).

273 Fig. 1 shows the TPC for various *R. alaternus* extracts according to the nature of the NaDES used
274 in comparison to ethanol. Whatever the plant part (*i.e.*, leaves, pods and roots), the extracts
275 obtained using NaDESs contains significantly more polyphenols (from 437.7 to 279.8 mg
276 GAE/100 g d.w.) than those obtained using ethanol (from 250 to 222.6 mg GAE/100 g d.w.).
277 Furthermore, the TPC are significantly more concentrated in leaves samples than in pods and

278 finally roots, whatever the solvents studied in this work. Such trend is the highest for ChCl-Gly
279 and the lowest for ethanol. Comparing the solvents efficiency on extraction process, the ChCl-
280 Gly is more efficient than other solvents studied in this work. In ChCl-Gly, the highest TPC values
281 are determined at 437.7, 361.3 and 317.0 mg GAE/100 g d.w. for leaves, pods and roots,
282 respectively. The ChCl-Gly is followed by ChCl-EG (TPC varying from 344.2 to 304.8 mg GAE/100
283 g d.w.) and ChCl-Ur (TPC varying from 346.2 to 279.8 mg GAE/100 g d.w.), for leaves, pods and
284 roots, respectively. The lowest amounts of polyphenols determined for the ethanol extracts are
285 of 250.0, 237.8 and 222.6 mg GAE/100 g d.w. for leaves, pods and roots, respectively. In
286 general, this highest extraction efficiency of TPC with NaDESs comparing with ethanol is related
287 to the H-bonding interactions between the polar compounds (polyphenols) and NaDESs as
288 previously reported (Duan et al., 2016). Duan and co-workers extracted polyphenols from
289 Chinese herbal using various deep eutectic solvents. Also, our results are in agreement with a
290 recent study reported in the literature by Islamčević Razboršek et al. (2020) in which the
291 extraction of phenolic compounds from *Aronia melanocarpa* plant using NaDESs is studied. This
292 work found higher extraction yield of phenolic compounds with NaDESs than those observed
293 with ethanol.

294 [Fig. 1]

295 **3.2. Determination of antioxidant capacity of the extracts**

296 **3.2.1. Radical scavenging activity**

297 **ABTS radical scavenging activity.** The capacity of extracts to scavenge ABTS free radicals was
298 evaluated in this study to determine extracts with high radical scavenging capacity (lowest IC₅₀
299 values). As shown in Fig. 2A, the NaDESs are more efficient than the ethanol, since they show

300 the lowest IC₅₀ values. The highest radical scavenging activity is detected with ChCl-Gly extracts
301 followed by ChCl-EG extracts > ChCl-Ur extracts > Ethanol extracts, respectively. When solvents
302 are compared, extracts obtained using ChCl-Gly are more active with an IC₅₀ values ranging
303 from 44.55 to 53.56 µg/mL, followed by ChCl-EG with IC₅₀ values varying from 55.47 to 58.71
304 µg/mL, and finally ChCl-Ur extracts with IC₅₀ ranging from 60.22 to 63.72 µg/mL. In comparison
305 to NaDESs solvents, ethanol extracts present the lowest efficiency in terms of radical
306 scavenging activity with an IC₅₀ values comprised within 66.28 and 69.56 µg/mL. Whatever the
307 plant's part (*i.e.*, leaves, pods or roots), the values of IC₅₀ and The Trolox Equivalent Antioxidant
308 Capacity (TEAC) values are close in the three parts (Fig. 2A, Table S2). These results confirm that
309 the phenolic compounds extracted from different parts of *R. alaternus* using NaDESs present an
310 excellent antioxidant activity.

311 [Fig 2.]

312 **DPPH radical scavenging activity.** The DPPH radical scavenging capacity was evaluated for the
313 different solvent extracts and for various parts of plant (leaves, pods and roots; Fig. 2B). The
314 IC₅₀ values range from 26.36 µg/mL for leaves extracted by ChCl-Gly (the highest activity) to
315 118.23 µg/mL for pods extracted using ChCl-Ur (the lowest activity) (Table S3). For ChCl-EG
316 extracts, the IC₅₀ values range from 51.36 to 68.81 µg/mL, with DPPH radical scavenging activity
317 rather similar to the ethanol extracts. On the whole, the IC₅₀ values increase in the following
318 order: ChCl-Gly extracts < ChCl-EG extracts < Ethanol extracts < ChCl-Ur extracts (Fig. 2B),
319 meaning the highest and the lowest DPPH radical scavenging activity are determined for the
320 ChCl-Gly and the ChCl-Ur extracts, respectively. All extracts obtained by using NaDESs present
321 stronger DPPH[•] radical scavenging capacity than those obtained with ethanol, except ChCl-Ur.

322 Our results are similar to the study of Oliveira and co-workers (Oliveira et al., 2021) on the
323 radical scavenging capacity of *Curcuma longa* L. plant. Former authors showed that *Curcuma*
324 *longa* L. leaves extracts were better hydroxyl radical scavengers after deep eutectic solvents
325 extraction notably for choline chloride / lactic acid (1:2) or choline chloride / acetic acid (1:2) in
326 comparison to ethanol, used as reference solvent.

327 **3.2.2. Reducing power**

328 The reducing power of a biological extract indicates its ability to reduce the Fe^{3+} /ferricyanide
329 complex to the Fe^{2+} form, which is checked by measuring the formation of Perl's Prussian blue
330 at 700 nm (Pan et al., 2010). The various NaDESs extracts from *R. alaternus* (*i.e.*, leaves, pods
331 and roots) were evaluated to determine the lowest EC_{50} values (Fig. 3, panel A). The nature of
332 solvent and the part of the plant also influence significantly the reducing capacity. The EC_{50}
333 values of the investigated extracts increases which mean the decreases of reducing power in
334 the following order: ChCl-Gly extracts > Ethanol extracts > ChCl-EG extracts > ChCl-Ur extracts.
335 Compared to other solvent extracts, the ChCl-Gly extracts give the highest reducing power,
336 corresponding to the lowest EC_{50} concentrations ranging from 77.53 to 85.05 $\mu\text{g}/\text{mL}$. The
337 lowest reducing power was obtained in ChCl-Ur extracts, with EC_{50} values ranging from 395.97
338 to 542.71 $\mu\text{g}/\text{mL}$ (Table S4). This analysis suggests that ChCl-Gly extracts are the most efficient
339 ones in terms of reducing power. While, according to the study of Ozturk and co-authors were
340 studied the extraction of polyphenols from orange peel waste using various DESs, this may be
341 related to a more efficient extraction of polyphenol compounds with this former NaDES (Ozturk
342 et al., 2018). Indeed, the reducing power of *R. alaternus* may be due to their TPC presence,

343 which may contribute to the antioxidant capacity by different reaction mechanisms (Duh et al.,
344 2001).

345 [Fig. 3]

346 3.2.3. Iron (II) chelating activity

347 Plant extracts enriched in polyphenols and other bioactive compounds may also present metal
348 chelation properties including iron chelating capacity; this latter activity plays a central role in
349 antioxidative mechanisms while inhibiting the formation of free radicals (Halliwell, 2007;
350 Yoshino & Murakami, 1998).

351 The capacity of *R. alaternus* extracts to chelate Fe^{2+} enabled the calculation of EC_{50} value, which
352 is the maximal effective concentration of extracts to obtain 50 % of iron chelating activity (Table
353 S5). The decrease of EC_{50} values meaning an increase the iron chelating activity. Hence, the
354 iron-chelation properties of the various extracts (Fig. 3, panel B) evolve in the following order:
355 ChCl-Gly extracts > Ethanol extracts > ChCl-EG extracts > ChCl-Ur extracts. The ChCl-Gly extracts
356 have the highest iron-chelating activity (smallest EC_{50}) with values ranging from 91.73 to 108.7
357 $\mu\text{g}/\text{mL}$. Besides, the ChCl-Ur exhibits the lowest iron-chelating properties with EC_{50} values
358 varying from 138.20 to 215.70 $\mu\text{g}/\text{mL}$. Furthermore, the Ethanol extracts chelate Fe^{2+} more
359 efficiently than ChCl-EG and ChCl-Ur ones. Hence, the ability of *R. alaternus* extracts to complex
360 iron is attributed to the presence of polyphenols, as previously reported in other plant study
361 (Oliveira et al., 2021). Indeed, extracts from various parts of *Curcuma longa* L. (i.e., leaves,
362 rhizome and flowers) present chelating properties after solid-liquid extraction carried out using
363 DESs choline chloride-based solvents or ethanol as reference. In accordance with $ABTS^{\circ+}$, DPPH

364 radical scavenging capacity and reducing power tests, ChCl-Ur is the less efficient solvent for
365 producing extracts endowed with the radical scavenging capacity and reducing capacity.

366 **3.3. Antimicrobial activity**

367 First, the antimicrobial activity of the solvents itself (Ethanol or NaDESs) was evaluated alone
368 against five bacterial strains of clinical interest. Although few MICs were obtained, in most
369 cases, the bacterial growth was affected by the solvents, even without reaching a MIC (Table
370 S6). Then, the microplates wells were gently homogenized and the OD540 was recorded. We
371 arbitrarily defined that a bacterial growth showing an optical density $OD \geq 75\%$ of the OD of
372 the growth control indicates few or no effect of the solvent. In some case, 1.3 % of the solvent
373 must be used as maximum value to avoid a negative effect on the bacterial growth (Table S7).
374 Our results also show the solvent negative effect on the bacterial growth in agreement with the
375 literature (Percevault et al., 2021). Former authors demonstrated that DES (choline chloride /
376 ethylene glycol (1:2) and betaine / citric acid (2:3)) are appropriate for the storage of bioactive
377 compounds to avoid microbial contamination, which could be promising for food and
378 pharmaceutical applications.

379 Therefore, the MIC of the extracts was determined against the bacteria with at most 1 % of
380 extract, in order to avoid any interference with the solvent activity. Only the extract of Roots-
381 ChCl-Gly showed a MIC at 0.5 % against *E. faecalis*. All other combinations showed no visible
382 effect. Hence, in a second series of experiments, higher concentrations of extracts (10, 5, 1, 0.5,
383 0.25 %) were investigated with care of the solvent potential effect (Table 1).

384 Among the Gram- bacteria, only the Leaves-ChCl-Ur extract has an effect on *E. coli* (MIC
385 reached at 10 % without solvent interference). In three cases (*i.e.*, Roots-ChCl-Gly vs. *P.*
386 *aeruginosa*, Leaves-ChCl-Ur and Pods-ChCl-Ur against *K. pneumoniae*), a MIC value of 10 % is
387 obtained, but at such concentration, the solvent fragilizes the cells (< 75 % of the growth
388 control determined for the solvent alone) and is thus, a result of impaired bacteria.

389 On the other hand, the two Gram+ bacteria are more sensitive to the extracts. The Leaves-ChCl-
390 Ur and Pods-ChCl-Gly extracts vs. *S. aureus*, and the Pods-Ethanol and Pods-ChCl-Gly extracts
391 vs. *E. faecalis* show a MIC at 10 %, yet with solvent interference. Only the extracts Leaves-
392 Ethanol, Leaves-ChCl-Gly and Leaves-ChCl-EG show no effect on *E. faecalis*. While, on all other
393 combinations, MICs are defined between 0.5 and 10 %, without solvent interference on this
394 strain.

395 Roots extracts (whatever the solvent) seems to have the best antimicrobial activity, with MICs
396 on both Gram+ bacteria (*i.e.*, *Staphylococcus aureus* and *Enterococcus faecalis*) without solvent
397 interference. This antimicrobial activity towards Gram+ bacteria may be related to their
398 absence of outer membrane, which could render them less resistant against extracts rich in
399 bioactive molecules compared to Gram- bacteria (Brul & Coote, 1999). Our results are in
400 agreement with Zhao and colleagues (Zhao et al., 2015), who found that extracts from *Sophora*
401 *japonica* using DESs choline chloride-based (*i.e.*, choline chloride / ethylene glycol (1:2), choline
402 chloride / glycerol (1:2) and choline chloride / urea (1:2)) presented antimicrobial properties
403 against gram+ bacteria (*Staphylococcus aureus*).

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409 **Table 1.** Minimum inhibitory concentrations of the extracts against the five bacterial strains.

Extract	Gram+		Gram-		
	<i>Staphylococcus aureus</i> (Sa)	<i>Enterococcus faecalis</i> (Ef)	<i>Pseudomonas aeruginosa</i> (Pa)	<i>Escherichia coli</i> (Ec)	<i>Klebsiella pneumoniae</i> (Kp)
Leaves-Ethanol	5% ⁺	≥ 10 %	≥ 10 %	≥ 10 %	≥ 10 %
Leaves-ChCl-Gly	5% ⁺	≥ 10 %	≥ 10 %	≥ 10 %	≥ 10 %
Leaves-ChCl-EG	5% ⁺	≥ 10 %	≥ 10 %	≥ 10 %	≥ 10 %
Leaves-ChCl-Ur	10% ⁺⁺	10% ⁺	≥ 10 %	10% ⁺	10% ⁺⁺
Pods-Ethanol	10% ⁺	10% ⁺	≥ 10 %	≥ 10 %	≥ 10 %
Pods-ChCl-Gly	10% ⁺⁺	10% ⁺⁺	≥ 10 %	≥ 10 %	≥ 10 %
Pods-ChCl-EG	5% ⁺	10% ⁺	≥ 10 %	≥ 10 %	≥ 10 %
Pods-ChCl-Ur	5% ⁺	5% ⁺	≥ 10 %	≥ 10 %	10% ⁺⁺
Roots-Ethanol	5% ⁺	5% ⁺	10 % [*]	≥ 10 %	≥ 10 %
Roots-ChCl-Gly	5% ⁺	0,5% ⁺	≥ 10 %	≥ 10 %	≥ 10 %
Roots-ChCl-EG	5% ⁺	5% ⁺	≥ 10 %	≥ 10 %	≥ 10 %
Roots-ChCl-Ur	5% ⁺	10% ⁺	≥ 10 %	≥ 10 %	≥ 10 %

410 (+): weak or no effect of the solvent; (++) : combined effect of the solvent; (*): strong effect of the

411 solvent; ≥ 10 %: no effect.

412 **3.4. Characterization of ChCl-Gly extracts by HPLC-MS/MS**

413 The qualitative determination of bioactive compounds extracted from three different extracts

414 of *R. alaternus* (i.e., leaves, pods and roots) with ChCl-Gly - corresponding to the best extract in

415 term of biological activities - were analyzed by HPLC-MS/MS (Table 2). Twenty-one compounds

416 were determined in the extracts. Among them, twelve molecules were newly identified in this
417 plant comparing to the literature (Nekkaa et al., 2021), including tyrosine (2.5 min),
418 dihydroquercetine pentoside glucoside (9.1 min), tryptophane (9.8 min), aloesin (11.3 min),
419 aloesol 7-glucoside (11.5 min), tectoridin (12.8 min), glucuronic acid (12.6/13.7 min), 7-hydroxy-
420 3-(2-hydroxypropyl)-5-methylisochromen-1-one (15.3 min), aloesol (15.4 min), piperonylidene
421 acetone (16.4 min), (R)-linalyl beta-vicianoside (18.1 min), neryl rhamnosyl-glucoside (18.3
422 min). Furthermore, six compounds *i.e.*, aloesin (11.3 min), aloesol glucoside (11.6 min), aloesol
423 7-glucoside (11.5 min), 7-hydroxy-3-(2 hydroxypropyl)-5-methylisochromen-1-one (15.3 min),
424 aloesol (15.4 min) and emodin (25.1 min) were common to the three extracts collected from
425 the various part of plant. Based on Table 2, the leaves provide the richest part of *R. alaternus* in
426 term of number of bioactive compounds identified in this extraction comparing to other parts,
427 and is followed by pods than roots. On the other hand, these finding evidences that the *R.*
428 *alaternus* plant presents total phenolic compounds with high antioxidant effect (*i.e.*,
429 dihydroquercetine pentoside glucoside, kaempferol, dihydroquercetin 3-O-glucoside,
430 kaempferide 3-glucoside and tectoridin), and organic acids (*i.e.*, malic acid, citric acid and
431 glucuronic acid), in addition to amino acids including tyrosine and tryptophan.

432 The ChCl-Gly (1:2) may have a better affinity towards bioactive compounds through hydrogen
433 bonding interactions, which would favour the antioxidant effects of extracts.

434 **4. Conclusion and Perspectives**

435 For the first time, three choline chloride-based NaDESs (choline chloride-glycerol (ChCl-Gly),
436 choline-chloride-ethylene glycol (ChCl-EG) and choline chloride-urea (ChCl-Ur)) were used for

437 the extraction of bioactive compounds from various parts of *R. alaternus* plant (*i.e.*, leaves,
438 pods and roots). Among all the studied extracts, the ChCl-Gly extracts from *R. alaternus*' leaves
439 presents the best content of total phenolic compounds and provides the highest antioxidant
440 activity compared to other investigated solvents such as NaDESs and ethanol, used in
441 reference. All NaDESs extracts show interesting antimicrobial activity with significant inhibitory
442 potential against Gram+ strains. Furthermore, the leaves, roots and pods extract carried out
443 using ChCl-Gly were characterized and quantified by HPLC-MS/MS, which confirms that the
444 phenolic compounds and flavonoids are the main antioxidant compounds. The *in vitro*
445 biological analysis of extracts suggests that ChCl-Gly is an efficient solvent for the extraction of
446 secondary metabolite from leaves, pods and roots of *R. alaternus*. These bio-based sustainable
447 and green processes present a great potential for pharmaceutical, cosmetic and food
448 applications.

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Table 2. Identification of bioactive compounds present in leaves, pods and roots of *R. alaternus* extracted using NaDES (ChCl-Gly) analysed by LC-MS/MS.

N°	Compound name	Molecular formula	tr (min)	[M + H] ⁻			[M + H] ⁺			plant part
				Measured m/z (Da)	Error (ppm)	MS/MS Fragmentation	Measured m/z (Da)	Error (ppm)	MS/MS Fragmentation	
1	Dihydroquercetine pentoside glucoside	C ₂₆ H ₃₀ O ₁₆	9.1	597.151	8.5	417.085 [M-hexose-H] ⁻ 285.038 [M-hexose - pentosyl - H] ⁻	599.162	1.6	n.d.	leaves
2	Kaempferol	C ₁₅ H ₁₀ O ₆	19.3	285.039	3.5	239.032 [M-HCOOH-H] ⁻ 229.049 [M-CO-CO-H] ⁻ 185.058 [M-CO-CO-CO ₂ +H] ⁺	287.056	3.5	285.052 [M-CHO+H] ⁺ 241.051 [M-HCOOH+H] ⁺ 231.072 [M-CO-CO+H] ⁺ 213.050 [M-CO-CO-H ₂ O+H] ⁺ 185.058 [M-CO-CO-H ₂ O-CO+H] ⁺	Pods
3	Dihydroquercetin 3-O-glucoside	C ₂₁ H ₂₂ O ₁₂	9.5	465.108	8.5	285.041 [M-hexose-2H] ⁻	467.116	8.5	305.065 [M-sucrose+H] ⁺ 181.014 [M-sucrose-C ₆ H ₄ CH] ⁺ 181.014 [C ₈ H ₅ O ₅] ⁺	leaves
4	Kaempferide 3-glucoside	C ₂₂ H ₂₂ O ₁₁	14.6	n.d.	n.d.	n.d.	463.125	4.3	301.069 [M-hexosyl+H] ⁺	Leaves, pods
5	Tectoridin	C ₂₂ H ₂₂ O ₁₁	12.8	461.118	19.5	n.d.	463.125	4.3	283.057 [M-hexose] ⁺ 255.063 [M-hexose-CO ₂] ⁺	Leaves, pods
6	Emodin	C ₁₅ H ₁₀ O ₅	25.1	269.045	3.7	241.047 [M-CO-H] ⁻ 255.053 [M-CO ₂ -H] ⁻ 210.029 [M-CO ₂ -CH ₃ -H] ⁻	271.058	7.3	229.046 [M-CH ₂ CO+H] ⁺ 225.051 [M-H ₂ O-CO+H] ⁺ 1978.059 [M-H ₂ O-2CO+H] ⁺	Leaves, pods, roots
7	Malic acid	C ₄ H ₆ O ₅	1.8	133.015	7.5	114.004 [M-H ₂ O-H] ⁻ 71.021 [M-H ₂ O-CO ₂ -H] ⁻	n.d.	n.d.	n.d.	Leaves, pods
8	Citric acid	C ₆ H ₈ O ₇	2.1	191.015	10.5	129.014 [M-H ₂ O-CO ₂ -H] ⁻ 111.007 [M-2H ₂ O-CO ₂ -H] ⁻ 87,011 [M-H ₂ O-CO ₂ -CH ₂ CO-H] ⁻	193.033	5.2	n.d.	Leaves, roots
9	Tyrosine	C ₉ H ₁₁ NO ₃	2.5	n.d.	n.d.	n.d.	182.080	5.5	165.083 [M-OH+H] ⁺ 135.077 [M-HCOOH+H] ⁺ 119.057 [M-HCOOH-NH ₃ +H] ⁺	Pods
10	Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	9.8	203.078	2.4	142.062 [M-CO ₂ -NH ₃ -H] ⁻ 116,050 [M-CO ₂ -NH ₃ -CN-H] ⁻	205.095	9.7	188.078 [M-NH ₃ +H] ⁺ 144.080 [M-NH ₃ -CO ₂ +H] ⁺ 118.068 [M-NH ₃ -CO ₂ -CN+H] ⁺	Leaves, pods
11	Aloesol 7-glucoside	C ₁₉ H ₂₄ O ₉	11.5	395.135	0.0	351.103 [M-C ₂ H ₄ O-H] ⁻ 231.063 [M-glycosyl-H] ⁻ 203.064 [M-glycosyl-CO-H] ⁻	397.149	0.0	233.077 [M-glycosyl+H] ⁺ 215.066 [M-glycosyl-H ₂ O + H] ⁺ 203.066 [M-glycosyl-CH ₂ O + H] ⁺	Leaves, pods, roots

12	7-hydroxy-3-(2-hydroxypropyl)-5-methylisochromen-1-one	C ₁₃ H ₁₄ O ₄	15.3	233.078	17	189.051 [M-CH ₃ COH-H] ⁻ 159.036 [M-CH ₃ COH-CH ₂ O-H] ⁻	235.095	4.2	217.077 [M-H ₂ O+H] ⁺ 191.068 [M-CH ₃ COH+H] ⁺ 176.046 [M-CH ₃ COH-CH ₃ +H] ⁺	Leaves, pods, roots
13	Piperonylidene acetone	C ₁₁ H ₁₀ O ₃	16.4	189.051	26	174.030 [M-CH ₃ -H] ⁻ 159.042 [M-CH ₂ COH-H] ⁻ 146.034 [M-CH ₂ COH-CH ₃ -H] ⁻	191.069	5.2	176.047 [M-CH ₃ +H] ⁺ 151.037 [M-H ₂ O-CH ₂ CO+H] ⁺ 148.050 [M-CH ₃ -CO+H] ⁺	Leaves, pods
14	(R)-Linalyl beta-vicianoside	C ₂₁ H ₃₆ O ₁₀	18.1	449.233	9.1	447.231 [M-H] ⁻ 315.179 [M-pentosyl-H] ⁻ 161.042 [M-pentosyl-C ₁₀ H ₁₇ OH-H] ⁻	471.216	8.5	333.079 [M-C ₁₀ H ₁₈ +Na] ⁺	leaves
15	Neryl rhamnosyl-glucoside	C ₂₂ H ₃₈ O ₁₀	18.3	507.251	16	461.246 [M-H] ⁻ 315.178 [M-Dehydrohexosyl-H] ⁻ 141.042 [M-Dehydrohexosyl-C ₁₀ H ₁₇ OH-H] ⁻	485.236	0.0	347.093 [M-C ₁₀ H ₁₈ +Na] ⁺ 204.040 [M-C ₁₀ H ₁₈ -dehydrohexosyl+Na] ⁺	leaves
16	Glucuronic acid	C ₁₆ H ₂₀ O ₁₀	12.6/1 3.7	371.098	0.2	249.062 [M-benzoic acid-H] ⁻ 175.019 [M-benzoic acid-glycerol-H] ⁻ 121.029 [benzoic acid-H] ⁻	373.111	5.3	n.d	leaves
17	Aloesol di glycoside	C ₂₅ H ₃₄ O ₁₄	10.3	n.d	n.d	n.d	559.202	0.0	397.151 [M-Hexosyl+H] ⁺ 233.074 [M-2Hexosyl+H] ⁺	Leaves, pods
18	Aloesin-glycoside	C ₂₅ H ₃₂ O ₁₄	10.1	555.180	13	n.d	557.189	3.6	395.139 [M-Hexosyl+H] ⁺ 233.078 [M-2Hexosyl+H] ⁺	pods
19	Aloesin	C ₁₉ H ₂₂ O ₉	11.3	393.121	5.1	273.079 [M-HOC ₆ H ₃ CO-H] ⁻ 231.061 [M-glycosyl - H] ⁻ 203.068 [M-glycosyl-CO - H] ⁻	395.135	3.5	233.077 [M-glycosyl + H] ⁺ 215.067 [M-glycosyl-H ₂ O + H] ⁺ 203.065 [M-glycosyl-CH ₂ O + H] ⁺	Leaves, pods, roots
20	Aloesol-glucoside	C ₁₉ H ₂₄ O ₉	11.6	395.135	2.5	275.089 [M-HOC ₆ H ₃ CO-H] ⁻ 231.066 [M-glycosyl - H] ⁻ 203.068 [M-glycosyl-CO - H] ⁻	397.151	6.0	233.077 [M-glycosyl + H] ⁺ 215.067 [M-glycosyl-H ₂ O + H] ⁺ 203.066 [M-glycosyl-CH ₂ O + H] ⁺	Leaves, pods, roots
21	Aloesol	C ₁₃ H ₁₄ O ₄	15.4	233.078	60	189.051 [M-C ₂ H ₄ O - H] ⁻	235.094	8.5	217.077 [M-H ₂ O+H] ⁺ 191.068 [M-C ₂ H ₄ O + H] ⁺ 176.047 [M-C ₂ H ₄ O-CH ₃ + H] ⁺	Leaves, pods, roots

454 n.d: not determined

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463 **References**

- 464 Ben Ammar, R., Kilani, S., Bouhlef, I., Skandrani, I., Naffeti, A., Boubaker, J., Ben Sghaier, M., Bhourri, W.,
465 Mahmoud, A., Chekir-Ghedira, L., & Ghedira, K. (2007). Antibacterial and cytotoxic activities of
466 extracts from (Tunisian) *Rhamnus alaternus* (Rhamnaceae). *Annals of Microbiology*, 57(3), 453–
467 460. <https://doi.org/10.1007/BF03175089>
- 468 Bhourri, W., Ben Sghaier, M., Kilani, S., Bouhlef, I., Dijoux-Franca, M. G., Ghedira, K., & Ghedira, L. C.
469 (2011). Evaluation of antioxidant and antigenotoxic activity of two flavonoids from *Rhamnus*
470 *alaternus* L. (Rhamnaceae): Kaempferol 3-O- β -isorhamninoside and rhamnocitrin 3-O- β -
471 isorhamninoside. *Food and Chemical Toxicology*, 49(5), 1167–1173.
472 <https://doi.org/10.1016/j.fct.2011.02.011>
- 473 Boussahel, S., Speciale, A., Dahamna, S., Ferlazzo, G., Harzallah, D., Amar, Y., Bonaccorsi, I., Cacciola, F.,
474 Cimino, F., Donato, P., & Cristani, M. (2015). Flavonoid profile, antioxidant and cytotoxic activity
475 of different extracts from Algerian *Rhamnus alaternus* L. bark. *Pharmacognosy Magazine*, 11(42),
476 102. <https://doi.org/10.4103/0973-1296.157707>
- 477 Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate
478 antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30.
479 [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- 480 Brul, S., & Coote, P. J. (1999). Preservative agents in foods mode of action and microbial resistance
481 mechanisms. *International Journal of Food Microbiology*, 50(1–2), 1–17.
482 [https://doi.org/10.1016/S0168-1605\(99\)00072-0](https://doi.org/10.1016/S0168-1605(99)00072-0)
- 483 Bugge, M., Hansen, T., & Klitkou, A. (2016). What is the bioeconomy? A review of the literature.
484 *Sustainability*, 8(7), 691. <https://doi.org/10.3390/su8070691>
- 485 Canabady-Rochelle, L. L. S., Harscoat-Schiavo, C., Kessler, V., Aymes, A., Fournier, F., & Girardet, J.-M.
486 (2015). Determination of reducing power and metal chelating ability of antioxidant peptides:
487 Revisited methods. *Food Chemistry*, 183, 129–135.
488 <https://doi.org/10.1016/j.foodchem.2015.02.147>
- 489 Catone, C. M., Ripa, M., Geremia, E., & Ulgiati, S. (2021). Bio-products from algae-based biorefinery on
490 wastewater: A review. *Journal of Environmental Management*, 293, 112792.
491 <https://doi.org/10.1016/j.jenvman.2021.112792>
- 492 Chanioti, S., & Tzia, C. (2018). Extraction of phenolic compounds from olive pomace by using natural
493 deep eutectic solvents and innovative extraction techniques. *Innovative Food Science &*
494 *Emerging Technologies*, 48, 228–239. <https://doi.org/10.1016/j.ifset.2018.07.001>

495 Dai, Y., van Spronsen, J., Witkamp, G.-J., Verpoorte, R., & Choi, Y. H. (2013). Natural deep eutectic
496 solvents as new potential media for green technology. *Analytica Chimica Acta*, 766, 61–68.
497 <https://doi.org/10.1016/j.aca.2012.12.019>

498 Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of*
499 *Agricultural and Food Chemistry*, 38(3), 674–677. <https://doi.org/10.1021/jf00093a019>

500 Duh, P.-D., Yen, G.-C., Yen, W.-J., & Chang, L.-W. (2001). Antioxidant effects of water extracts from barley
501 (*Hordeum vulgare* L.) prepared under different roasting temperatures. *Journal of Agricultural*
502 *and Food Chemistry*, 49(3), 1455–1463. <https://doi.org/10.1021/jf000882l>

503 Eloff, J. (1998). A sensitive and quick microplate method to determine the minimal inhibitory
504 concentration of plant extracts for bacteria. *Planta Medica*, 64(08), 711–713.
505 <https://doi.org/10.1055/s-2006-957563>

506 Halliwell, B. (2007). Biochemistry of oxidative stress. *Biochemical Society Transactions*, 35(5), 1147–1150.
507 <https://doi.org/10.1042/BST0351147>

508 Islamčević Razboršek, M., Ivanović, M., Krajnc, P., & Kolar, M. (2020). Choline chloride based natural
509 deep eutectic solvents as extraction media for extracting phenolic compounds from chokeberry
510 (*Aronia melanocarpa*). *Molecules*, 25(7), 1619. <https://doi.org/10.3390/molecules25071619>

511 ISO 20776-1, :2019. (2019). *Susceptibility testing of infectious agents and evaluation of performance of*
512 *antimicrobial susceptibility test devices—Part 1: Broth micro-dilution reference method for*
513 *testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria*
514 *involved in infectious diseases*. 2019. [https://www.iso.org/obp/ui/#iso:std:iso:20776:-1:ed-2:](https://www.iso.org/obp/ui/#iso:std:iso:20776:-1:ed-2:v1:en)
515 [v1:en](https://www.iso.org/obp/ui/#iso:std:iso:20776:-1:ed-2:v1:en).

516 Jeong, K. M., Zhao, J., Jin, Y., Heo, S. R., Han, S. Y., Yoo, D. E., & Lee, J. (2015). Highly efficient extraction
517 of anthocyanins from grape skin using deep eutectic solvents as green and tunable media.
518 *Archives of Pharmacal Research*, 38(12), 2143–2152. [https://doi.org/10.1007/s12272-015-0678-](https://doi.org/10.1007/s12272-015-0678-4)
519 [4](https://doi.org/10.1007/s12272-015-0678-4)

520 Longo, L., Vasapollo, G., & Rescio, L. (2005). Identification of anthocyanins in *Rhamnus alaternus* L.
521 berries. *Journal of Agricultural and Food Chemistry*, 53(5), 1723–1727.
522 <https://doi.org/10.1021/jf048253p>

523 Moussi, K., Nayak, B., Perkins, L. B., Dahmoune, F., Madani, K., & Chibane, M. (2015). HPLC-DAD profile
524 of phenolic compounds and antioxidant activity of leaves extract of *Rhamnus alaternus* L.
525 *Industrial Crops and Products*, 74, 858–866. <https://doi.org/10.1016/j.indcrop.2015.06.015>

526 Nagarajan, J., Krishnamurthy, N. P., Nagasundara Ramanan, R., Raghunandan, M. E., Galanakis, C. M., &
527 Ooi, C. W. (2019). A facile water-induced complexation of lycopene and pectin from pink guava
528 byproduct: Extraction, characterization and kinetic studies. *Food Chemistry*, *296*, 47–55.
529 <https://doi.org/10.1016/j.foodchem.2019.05.135>

530 Nekkaa, A., Benaissa, A., Mutelet, F., & Canabady-Rochelle, L. (2021). *Rhamnus alaternus* plant:
531 Extraction of bioactive fractions and evaluation of their pharmacological and phytochemical
532 properties. *Antioxidants*, *10*(2), 300. <https://doi.org/10.3390/antiox10020300>

533 Oliveira, G., Marques, C., de Oliveira, A., de Almeida dos Santos, A., do Amaral, W., Ineu, R. P., Leimann,
534 F. V., Peron, A. P., Igarashi-Mafra, L., & Mafra, M. R. (2021). Extraction of bioactive compounds
535 from *Curcuma longa* L. using deep eutectic solvents: *In vitro* and *in vivo* biological activities.
536 *Innovative Food Science & Emerging Technologies*, *70*, 102697.
537 <https://doi.org/10.1016/j.ifset.2021.102697>

538 Oyaizu, M. (1986). Studies on products of browning reaction. Antioxidative activities of products of
539 browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*,
540 *44*(6), 307–315. <https://doi.org/10.5264/eiyogakuzashi.44.307>

541 Ozturk, B., Parkinson, C., & Gonzalez-Miquel, M. (2018). Extraction of polyphenolic antioxidants from
542 orange peel waste using deep eutectic solvents. *Separation and Purification Technology*, *206*, 1–
543 13. <https://doi.org/10.1016/j.seppur.2018.05.052>

544 Palos-Hernández, A., Gutiérrez Fernández, M. Y., Escuadra Burrieza, J., Pérez-Iglesias, J. L., & González-
545 Paramás, A. M. (2022). Obtaining green extracts rich in phenolic compounds from
546 underexploited food by-products using natural deep eutectic solvents. Opportunities and
547 challenges. *Sustainable Chemistry and Pharmacy*, *29*, 100773.
548 <https://doi.org/10.1016/j.scp.2022.100773>

549 Pan, Y., He, C., Wang, H., Ji, X., Wang, K., & Liu, P. (2010). Antioxidant activity of microwave-assisted
550 extract of *Buddleia officinalis* and its major active component. *Food Chemistry*, *121*(2), 497–502.
551 <https://doi.org/10.1016/j.foodchem.2009.12.072>

552 Percevault, L., Limanton, E., Nicolas, P., Paquin, L., & Lagrost, C. (2021). Electrochemical determination
553 and antioxidant capacity modulation of polyphenols in deep eutectic solvents. *ACS Sustainable*
554 *Chemistry & Engineering*, *9*(2), 776–784. <https://doi.org/10.1021/acssuschemeng.0c07023>

555 Quideau, S., Deffieux, D., Douat-Casassus, C., & Pouységu, L. (2011). Plant polyphenols: Chemical
556 properties, biological activities, and synthesis. *Angewandte Chemie International Edition*, *50*(3),
557 586–621. <https://doi.org/10.1002/anie.201000044>

558 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity
559 applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and*
560 *Medicine*, 26(9–10), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)

561 Si, X., Chen, Q., Bi, J., Wu, X., Yi, J., Zhou, L., & Li, Z. (2016). Comparison of different drying methods on
562 the physical properties, bioactive compounds and antioxidant activity of raspberry powders:
563 Effect of drying method on properties of raspberry powders. *Journal of the Science of Food and*
564 *Agriculture*, 96(6), 2055–2062. <https://doi.org/10.1002/jsfa.7317>

565 Sillero, L., Prado, R., Welton, T., & Labidi, J. (2021). Extraction of flavonoid compounds from bark using
566 sustainable deep eutectic solvents. *Sustainable Chemistry and Pharmacy*, 24, 100544.
567 <https://doi.org/10.1016/j.scp.2021.100544>

568 Singleton, V. L., & Rossi, J. A. (1965). *Colorimetry of total phenolics with phosphomolybdic-*
569 *phosphotungstic acid reagents*. 16(3), 144–158.

570 Tacherfiout, M., Petrov, P. D., Mattonai, M., Ribechini, E., Ribot, J., Bonet, M. L., & Khettal, B. (2018).
571 Antihyperlipidemic effect of a *Rhamnus alaternus* leaf extract in Triton-induced hyperlipidemic
572 rats and human HepG2 cells. *Biomedicine & Pharmacotherapy*, 101, 501–509.
573 <https://doi.org/10.1016/j.biopha.2018.02.106>

574 Tang, W., & Row, K. H. (2020). Design and evaluation of polarity controlled and recyclable deep eutectic
575 solvent based biphasic system for the polarity driven extraction and separation of compounds.
576 *Journal of Cleaner Production*, 268, 122306. <https://doi.org/10.1016/j.jclepro.2020.122306>

577 Trivedi, T. J., Lee, J. H., Lee, H. J., Jeong, Y. K., & Choi, J. W. (2016). Deep eutectic solvents as attractive
578 media for CO₂ capture. *Green Chemistry*, 18(9), 2834–2842.
579 <https://doi.org/10.1039/C5GC02319J>

580 Vilková, M., Płotka-Wasyłka, J., & Andruch, V. (2020). The role of water in deep eutectic solvent-base
581 extraction. *Journal of Molecular Liquids*, 304, 112747.
582 <https://doi.org/10.1016/j.molliq.2020.112747>

583 Wan Mahmood, W. M. A., Lorwirachsutee, A., Theodoropoulos, C., & Gonzalez-Miquel, M. (2019).
584 Polyol-based deep eutectic solvents for extraction of natural polyphenolic antioxidants from
585 *Chlorella vulgaris*. *ACS Sustainable Chemistry & Engineering*, 7(5), 5018–5026.
586 <https://doi.org/10.1021/acssuschemeng.8b05642>

587 Wei, Z., Qi, X., Li, T., Luo, M., Wang, W., Zu, Y., & Fu, Y. (2015). Application of natural deep eutectic
588 solvents for extraction and determination of phenolics in *Cajanus cajan* leaves by ultra

589 performance liquid chromatography. *Separation and Purification Technology*, 149, 237–244.
590 <https://doi.org/10.1016/j.seppur.2015.05.015>

591 Yoshino, M., & Murakami, K. (1998). Interaction of iron with polyphenolic compounds: Application to
592 antioxidant characterization. *Analytical Biochemistry*, 257(1), 40–44.
593 <https://doi.org/10.1006/abio.1997.2522>

594 Zhang, L., Ravipati, A. S., Koyyalamudi, S. R., Jeong, S. C., Reddy, N., Smith, P. T., Bartlett, J., Shanmugam,
595 K., Münch, G., & Wu, M. J. (2011). Antioxidant and anti-inflammatory activities of selected
596 medicinal plants containing phenolic and flavonoid compounds. *Journal of Agricultural and Food*
597 *Chemistry*, 59(23). <https://doi.org/10.1021/jf203146e>

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615 **Figure captions**

616 **Fig. 1.** Total phenolic content (TPC) of *R. alaternus*'s leaves, pods and roots extracted with
617 natural deep eutectic solvents (NaDESs: ChCl-Gly, ChCl-EG, ChCl-Ur) and compared to Ethanol as
618 reference.

619 **Fig. 2.** Radical scavenging activity of different parts from *R. alaternus* extracted using natural
620 deep eutectic solvents (NaDESs): ChCl-Gly, ChCl-EG, ChCl-Ur in reference to Ethanol. (A) ABTS
621 test and (B) DPPH test.

622 **Fig. 3.** Reducing activity (A) and Iron chelating capacity (B) of different parts from *R. alaternus*
623 extracted using natural deep eutectic solvents (NaDESs); ChCl-Gly, ChCl-EG, ChCl-Ur and
624 Ethanol.

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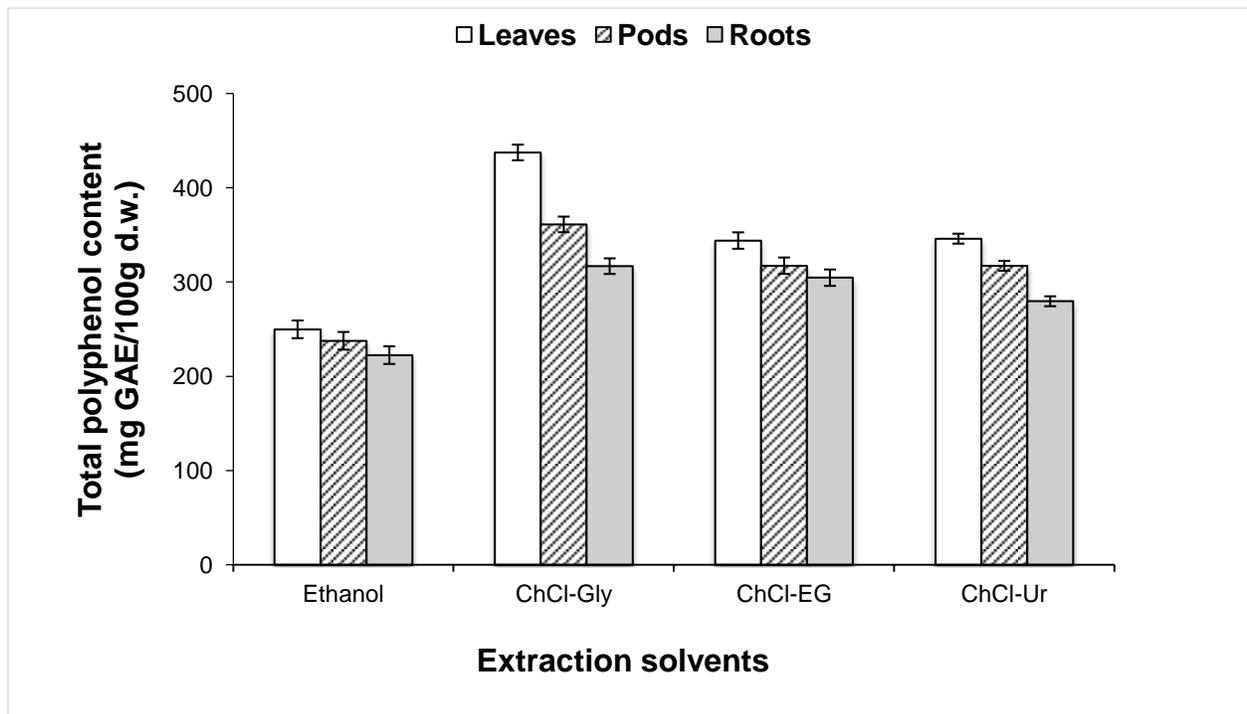
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636 **Figure graphics**

637 **Fig. 1**



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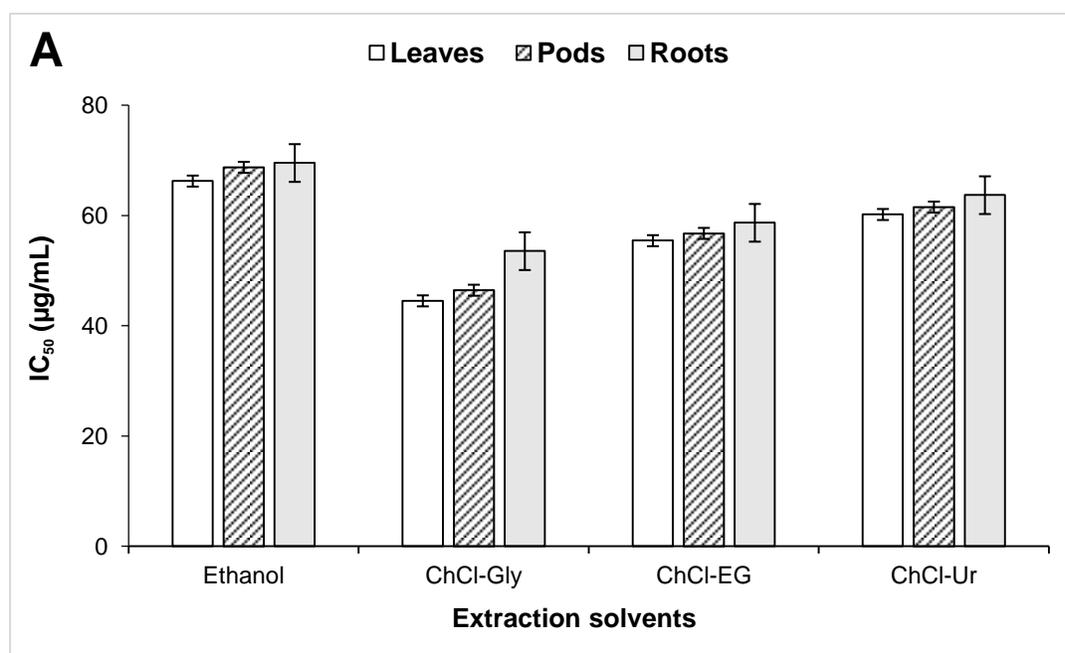
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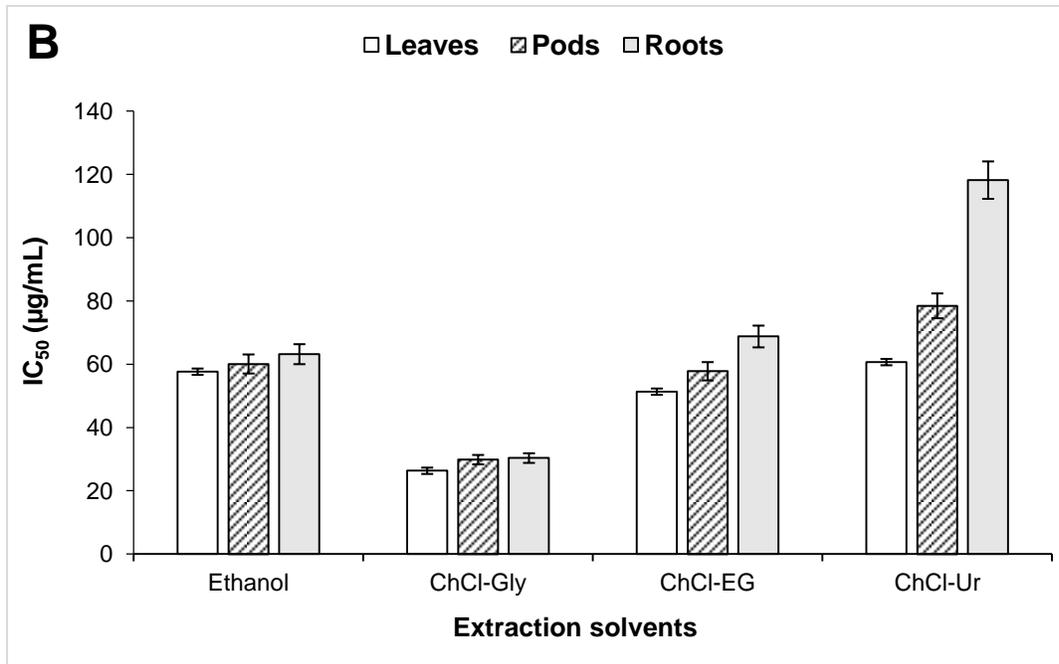
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647 **Fig. 2**



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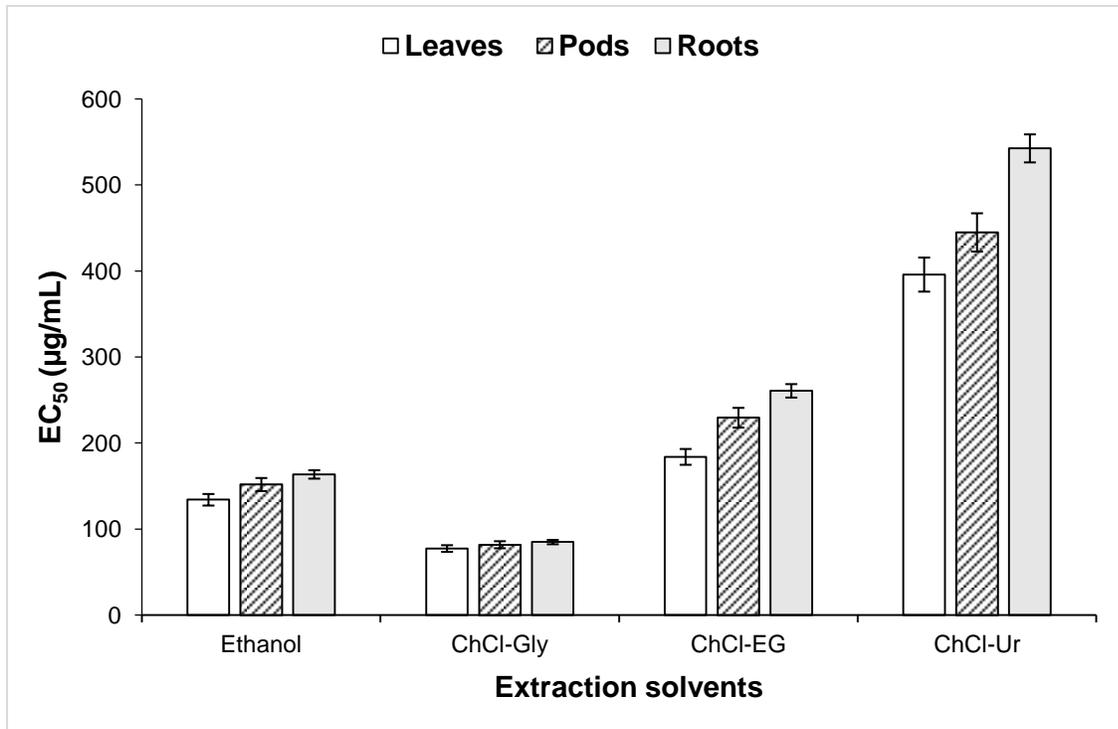
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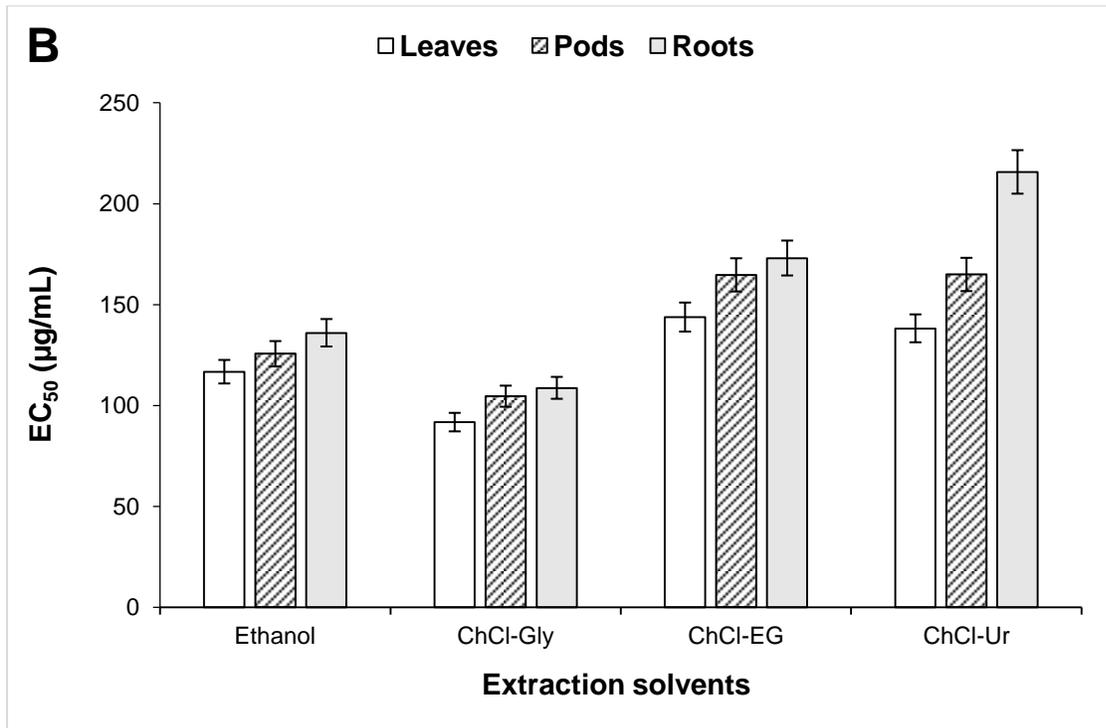
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652 **Fig. 3**

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