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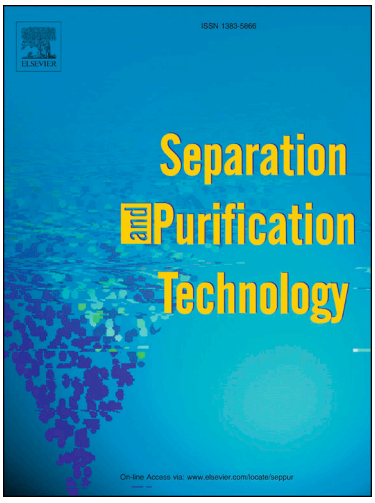

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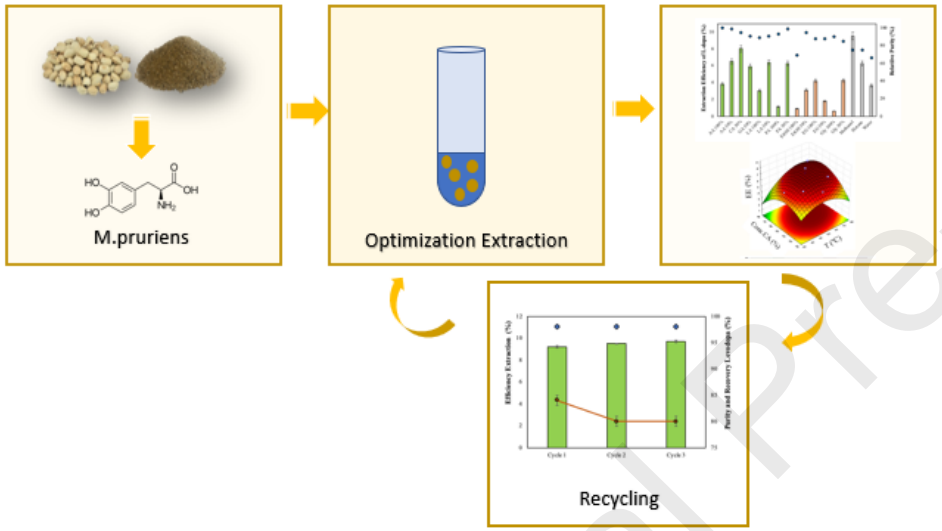
Highlights

- Aqueous solutions of organic acids are promising substitutes for organic solvents.

- Performance in the extraction of L-dopa from the seeds of *M. Pruriens* was assessed.

- High purity and yields of L-dopa have been achieved with the investigated compounds.

Graphical Abstract



Aqueous Solutions of Organic Acids as Effective Solvents for Levodopa Extraction from *Mucuna pruriens* Seeds

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Abstract: Levodopa is an amino acid commonly used in the treatment of Parkinson's disease found in several plants, such as *Mucuna pruriens*. The extraction of levodopa from biomass has been achieved using methanol, ethanol:water mixtures in presence of ascorbic acid, chloroform in alkaline media, and acetonitrile. Aiming at finding more sustainable solvents and develop efficient extraction processes, in this work, aqueous solutions of carboxylic acids (acetic, propionic, citric, glycolic, and lactic acid) and (poly)alcohols (ethanol, ethylene glycol and glycerol) were studied for the extraction of levodopa from *Mucuna pruriens* seeds. An initial screening with aqueous solutions of these compounds (at 50 wt.%) was conducted at 50 °C, with an extraction time of 90 minutes at a solid/liquid (biomass/solvent) ratio of 1:10. Based on these results, citric acid aqueous solutions were identified as the best solvent, and an experimental design was carried out to optimize the temperature (T), solid/liquid ratio (S:L) and concentration of acid (wt.%), with the following optimal extraction conditions found: T = 60 °C, S:L = 1:7 and concentration of acid at 58 wt.%. Under these optimal conditions, an extraction efficiency of 9.2 ± 0.1 wt.% of levodopa was achieved. The recovery of levodopa from the acidic aqueous solution was achieved using an ion exchange column, allowing the recovery of approximately 84% of levodopa. The solvent was shown to be reusable in three successive extraction cycles, with no significant losses in the extraction efficiency of levodopa. The results here obtained show that citric acid aqueous solutions can lead to the effective extraction of levodopa from seeds of *Mucuna pruriens*, serving as basis for the development of more effective and environmentally friendly processes to recover natural products with therapeutic properties.

Keywords: levodopa, solid-liquid extraction, co-solvency, green solvent, organic acids.

1.Introduction

Mucuna pruriens is an important plant of the Fabaceae family, whose seeds present a high concentration of Levodopa (L-dopa).[1] L-dopa is an amino acid widely used in the treatment of neurodegenerative disorders, such as Parkinson's disease,[2] considered the second most common neurodegenerative disorder in the world, behind Alzheimer's disease, affecting approximately 0.3% of the world's population.[3,4]

L-Dopa was first synthesized in 1911 and since then has been presented as promising drug in the treatment of progressive neurodegenerative diseases.[5] Despite presenting side effects such as involuntary movements, the pharmacokinetic and pharmacokinetic activities of L-dopa as well as the ability to combat neurodegenerative disorders has generated interest for the scientific community.[6–8] L-dopa is present in high content in *M. pruriens* seeds, thus boosting the interest in exploring biomass as a natural source of this compound.

Volatile organic solvents such as methanol, hexane and chloroform are often used in solid-liquid extraction processes due to their high extraction capacity.[9–11] However, they present intrinsic problems such as toxicity, high volatility and low selectivity, with a consequent loss of process efficiency due to the need of further purification strategies.[12,13] In particular, the extraction of L-dopa from *M. pruriens* seeds has been attempted using methanol, resulting in an extract containing 12.16 wt.% of L-dopa.[10] Mixtures of ethanol:water in presence of ascorbic acid, and chloroform in alkaline media were also investigated, and extraction yields of 1.78 and 4 wt.% were reported.[11] Rane *et al*. used a mixture of acetonitrile and methanol under ultrasounds to extract L-dopa from seeds of different types of *M. pruriens*, reporting a yield of levodopa of 7.61 wt.%.[14]

Although the use of volatile organic solvents has allowed relatively high L-dopa yields, there is a growing interest in developing innovative, efficient, sustainable and low-cost extraction processes based on alternative solvents. Most of these works address compounds or solvents such as ionic liquids, eutectic mixtures, surfactants, and more recently green hydrotopes.[12,15,16] However, the use of low-cost compounds such as polycarboxylic acids or polyalcohols and their aqueous solutions is poorly investigated. Herein, their use in aqueous solutions for the extraction of L-dopa is studied as both a cost-effective option to alternative solvents and as a form to reduce the environmental impact generated by conventional solvents.[17] Moreover, most of the compounds here studied are approved ingredients or solvents used in the food, pharmaceutical and cosmetic industries.[18,19]

In this work, the potential of aqueous solutions of carboxylic acids (acetic, propionic, citric, glycolic, and lactic acid) and (poly)alcohols (ethanol, ethylene glycol and glycerol) to selectively extract L-dopa from *M. pruriens* seeds was evaluated aiming at developing a sustainable process. After the screening of the best extracting solvent at fixed operational conditions, the extraction yield was optimized through a response surface methodology (RSM) by changing the following variables: extraction temperature (T), solid-liquid ratio (S:L) and concentration of solvent (C, wt.%). Finally, the recovery of L-dopa from the extracts and the reuse of aqueous solutions were also demonstrated.

2. Materials and Methods

2.1 Biomass.

M. pruriens seeds were purchased by a company specialized in grains located in the São Paulo region, Brazil. The samples supplied in dry form were reduced to a granulometry of 40-20 mesh vacuum-packed and protected from light until use.

2.2 Reagents, Standards and Solvents.

Double distilled deionized water was obtained from a Millipore Milli-Q water purification system (Millipore, USA). The amino acid standard 3,4-Dihydroxy-L-phenylalanine or L-Dopa (99.9 wt%) was obtained commercially by Sigma Aldrich (Germany). The molecular structure of all solvents investigated in this work and of L-Dopa are shown in Figure 1. A description of the supplier and purity of the compounds acetic acid, citric acid, glycolic acid, lactic acid, propionic acid, ethanol, ethylene glycol, and glycerol is provided in Table 1. All other chemicals were of analytical quality and purchased from common sources.

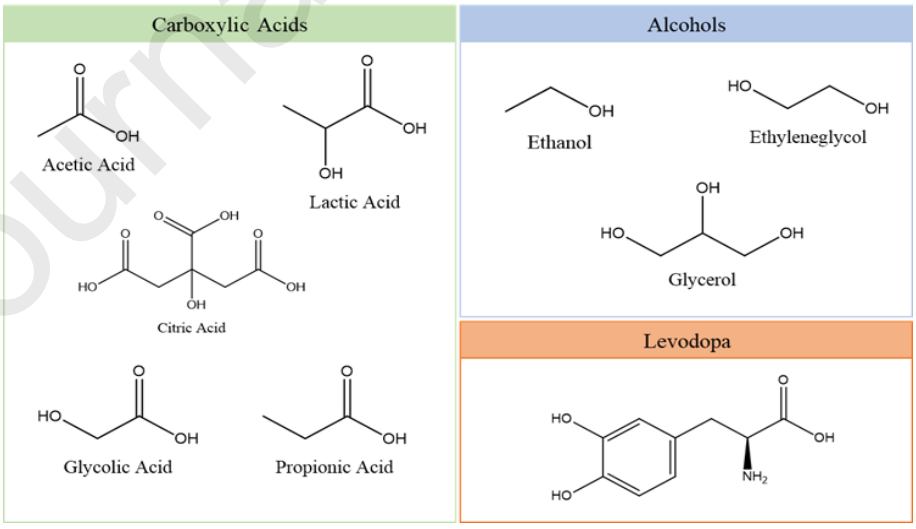

Figure 1. Chemical structures of levodopa and carboxylic acids/(poly)alcohols investigated.

Table 1. Compounds Description, Molecular formula, CAS Number, Purity and Supplier.

Compound	Molecular formula	CAS number	Purity/wt %	Supplier
Acetic acid (AA)	$C_2H_4O_2$	64-19-7	99.9	Honeywell
Citric acid (CA)	$C_6H_8O_7$	77-92-9	99.5	Panreac
Glycolic acid (GA)	$C_2H_4O_3$	79-14-1	99.0	Acros Organics
Lactic acid (LA)	$C_3H_6O_3$	10326-41-7	90.0	Sigma Aldrich
Propionic acid (PA)	$C_3H_6O_2$	79-09-4	99.0	Acros Organics
Ethylene glycol (EG)	$C_2H_6O_2$	107-21-1	99.5	Sigma Aldrich
Glycerol (G)	$C_3H_8O_3$	56-81-5	99.8	Fisher Chemical
Hexane	C_6H_{14}	110-54-3	99.0	Carlo Erba
Methanol	CH_4O	67-56-1	HPLC	Fisher Scientific

2.3 Solid-liquid extraction and solvent screening.

An initial screening of aqueous solutions of carboxylic acids and polyols was carried out, whose concentrations studied are summarized in Table 2. Different concentrations were prepared to assess the selectivity of each solvent in the levodopa extraction process. The solid-liquid extractions were performed using a Carousel 12 Plus ™ reaction station (Radleys Tech), with temperature and agitation control and coupling system to avoid solvent loss. The extractions were performed with magnetic stirring (300 rpm) and at a temperature of 50 ° C (± 0.5 ° C), time of 90 min and S:L ratio of 1:10. After the extraction, suspensions were centrifuged and the supernatant was filtered using a 0.20 μm syringe filter. A 20 μL aliquot was collected and added to 4980 μL of Milli-Q water. All results presented are based on three independent experiments and expressed as average ± standard deviation.

Table 2 – List of carboxylic acids and (poly)alcohols investigated, abbreviations and concentrations used in the initial screening.

Compounds	Abbreviation	Concentration (wt.%)
Acetic Acid	(AA)	50 100
Citric Acid	(CA)	50
Glycolic Acid	(GA)	50
Lactic Acid	(LA)	50 100
Propionic Acid	(PA)	50 100
Glycerol	(Gly)	50 100
Ethanol	(EtOH)	25 50 75 100
Ethylene Glycol	(EG)	50 100

2.4 Response Surface Methodology.

After the identification of the most suitable solvent, with the highest L-dopa extraction efficiency and selectivity, an optimization of the solvent concentration and other operational conditions was performed. The optimization was performed through a central composite planning using the response surface methodology 2^3, with 5 central point assays, corresponding to a total of 19 extraction experiments at each set of conditions. The independent variables were extraction temperature (T), solid-liquid (biomass-solvent) ratio S:L, concentration of solvent (C, wt.%), whereas extraction efficiency of L-dopa ($EE_{levo,}$ %) was considered as a dependent variable. The surface responses were plotted by changing two variables within the experimental ranges. The results were considered statistically significant, with a 95 % confidence interval ($p < 0.05$).

147 *2.5 Quantification of L-Dopa.*

148 To quantify L-Dopa, DAD-HPLC analysis were performed based on the configuration
149 of the analytical column C18 (250 × 4.60 mm, Kinetex 5 µm C18 100 A) from
150 Phenomenex. The column temperature was adjusted to 30 °C. The mobile phase
151 consisted of 89.9 % (v/v) % acetonitrile, 10 (v/v) % water and 0.1 (v/v) % acetic acid
152 (AA). The separations were performed in the isocratic mode, at a flow rate of 0.8
153 mL/min. Detection was performed at a wavelength of 280 nm. Data acquisition and
154 evaluation were performed based on a previously established calibration curve (R^2 =
155 0.9987). The extraction efficiency of levodopa (EE_{levo}, %) was determined by equation
156 1. The relative purity of L-dopa was obtained by dividing the area of the levodopa
157 HPLC peak by the total area of all peaks present at 280 nm.

158 $$EE_{levo}\% = \frac{w_{levo}}{w_{biomass}} . 100 \quad (1)$$

159 where w_{levo} corresponds to the L-Dopa weight present in the filtered solution after
160 solid-liquid extraction and $w_{biomass}$ is the initial biomass mass.

161

162 *2.6 Recovery of levodopa from the aqueous extract.*

163 After identifying the ideal conditions for L-dopa extraction, its recovery from the
164 aqueous media was investigated. The methodology used consisted of an ion exchange
165 separation column filled with Dowex 500W X8 resin. First, the column was pre-treated
166 with 2 mL of methanol, followed by 2 mL of a concentrated solution of HCl (pH = 1).
167 After extraction, the aqueous solutions containing levodopa were adjusted to pH 1 with
168 0.1 M HCl to obtain protonated L-Dopa. Approximately 2 mL of aqueous solution
169 containing L-dopa was passed through the column, followed by the elution of 2 mL of
170 deionized water to recover the retained L-dopa. Finally, the column was regenerated

with 5 mL of methanol for successive applications. The fractions of each step were recovered and analysed by DAD-HPLC. The recovery efficiency ($RE_{levo,}$ %) of L-dopa was determined by equation 2.

$$RE_{levo}\% = \frac{w_{Lf}}{w_{Li}}.100 \quad (2)$$

where w_{Lf} is the weight of recovered L-dopa after elution and w_{Li} is the weight of L-dopa in the initial extract.

2.7 Nuclear magnetic resonance (NMR).

The aqueous solutions of recovered solvent and the fractions obtained after the recovery of L-dopa were analyzed by ^{1}H and ^{13}C NMR, using a Bruker Avance 300 (France) at 300.13 MHz and 75.47 MHz, respectively, and deuterium oxide (D_2O) as a solvent and trimethylsilylpropanoic acid (TSP) as an internal standard. These samples were then compared with pristine L-Dopa and chemical grade solvent to ascertain their purity and stability.

3. Results and Discussion

In this work we aim to develop a solvent and sustainable process to extract *L*-Dopa from biomass, in which aqueous solutions of carboxylic acids and (poly) alcohols were investigated as low cost and biocompatible alternative solvents. After an initial screening to identify the most promising solvent, several process variables were studied, namely temperature (T), solid-liquid ratio (S:L) and concentration of solvent (C, wt.%).

3.1 Solvent screening

Aqueous solutions of carboxylic acids and (poly)alcohols were initially investigated to identify the solvent with the best extraction performance and selectivity to L-dopa from *M. pruriens* seeds. The aqueous solutions studied were prepared at the concentrations

shown in Table 2. Carboxylic acids and (poly)alcohols that are liquid at 25 °C were also investigated in their pure form. For comparison purposes, fixed operational conditions were used, these being a 1:10 S:L ratio, an extraction time of 90 min and a temperature of 50 °C. The screening results obtained in terms of extraction efficiency of levodopa and selectivity (given by the relative purity) are shown in Figure 2.

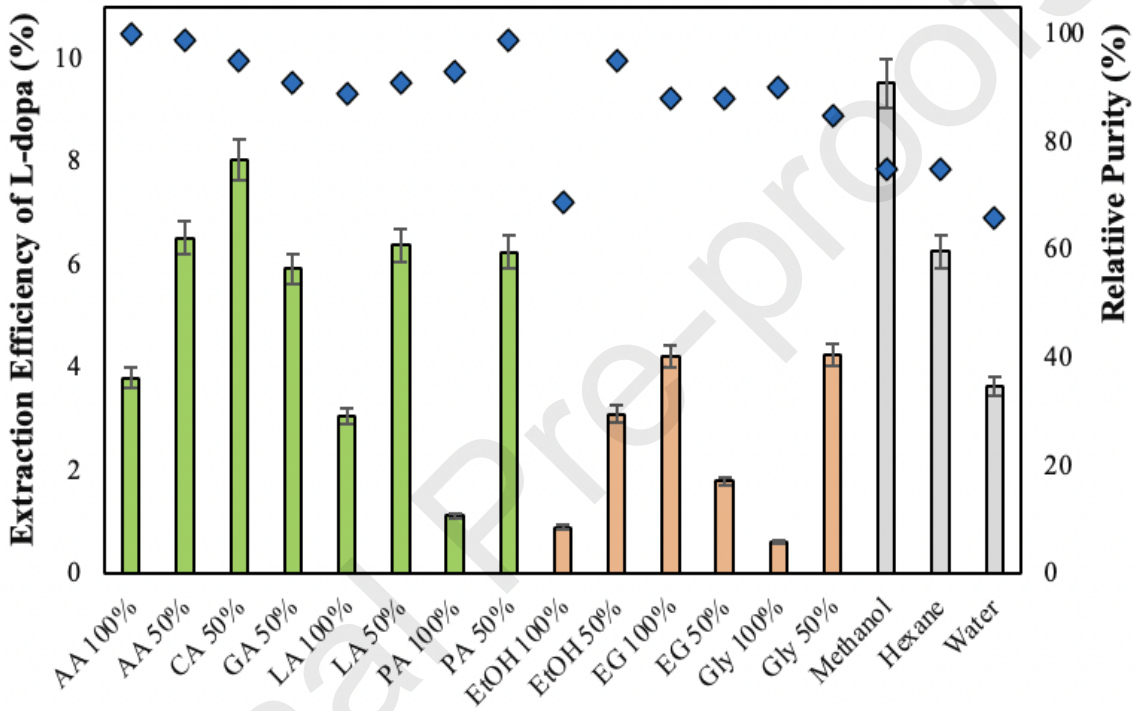

Figure 2. Screening of aqueous solutions of (■) carboxylic acids, (■) (poly)alcohols and (■) methanol, hexane and water to extract levodopa, given by the extraction efficiency (bars). Relative purity of levodopa (◆). Extraction performed at fixed conditions: T = 50°C, S:L ratio = 1:10, time = 90 min.

The *L*-dopa extraction efficiency ranges from 0.6 to 8.0 wt.% with the solvents investigated. When analysing the data set for pure carboxylic acids for those that are liquid at 25ºC, extraction yields of 3.8 ± 0.2 wt.%, 3.1 ± 0.4 wt.%, 1.1 ± 0.4 wt.% are found for acetic acid (AA), lactic acid (LA) and propionic acid (PA), respectively.

These results show that the dissociation constant (pK_a) of these acids,[20] all of them comprising one carboxylic group, is not responsible for the extraction performance of L-dopa (acetic acid (pK_a = 4.76); lactic acid (pKa = 3.86), propionic acid (pK_a = 4.88)). It seems however that the chemical structure and a shorter alkyl chain is preferable to enhance the extraction of acetic acid, as shown by the higher performance of pure acetic acid. Despite these differences, overall the pure acids are not as efficient as their aqueous solutions in the extraction of L-dopa.

For all acids investigated, the respective aqueous solutions show a superior extraction capacity of levodopa, which may be associated with a co-solvency phenomenon. Co-solvents have the ability of improving solubility, and consequently the extraction efficiency of target compounds from biomass.[21] This effect is evident in mixtures of AA at 50 wt.% (6.5 ± 0.3 wt.%), LA at 50 wt.% (6.4 ± 0.5 wt.%), and PA 50 wt.% (6.3 ± 0.3 wt.%). On the other hand, pure water leads to an extraction efficiency of levodopa of 3.6 ± 0.3 wt.%. Overall, mixtures consisting of (water + acid) have higher physical (e.g. lower viscosity) and solvation properties than their individual constituents, as reported in the literature for other co-solvents.[22] Even with extraction yields lower than those obtained with aqueous solutions of organic acids, a similar effect of co-solvency is noticeable in extractions performed with glycerol, e.g. when comparing pure Gly (0.6 ± 0.1 wt.%) and Gly at 50 wt.% (4.3 ± 0.2 wt.%).

An important aspect observed with the studied aqueous solutions is the extraction selectivity. Aqueous solutions of carboxylic acids lead to relative purities of levodopa higher than 90%. The selectivity of the extraction is associated with the intermolecular interactions that occur between the solvent and the compound of interest, which is directly associated with the polarity of the molecular species.[23] Although neat ethanol is often used in solid-liquid extractions, leading to high extraction efficiencies, it does

not present an effective selectivity when compared to aqueous solutions of organic acids. Also with ethanol, its mixture with water leads to higher extraction efficiencies of levodopa, revealing the co-solvency effect.

Methanol and hexane are solvents typically used in the extraction of levodopa from biomass, and were here investigated. Both solvents allow high yields in the extraction of levodopa (9.5 and 6.3 wt.%), but both present poor selectivity when compared with the aqueous solutions of organic acids here studied.

Overall, from the initial screening on several solvents to extract levodopa, aqueous solutions of citric acid appear as the ones leading to higher extraction efficiencies and higher relative purity of levodopa (95 %), being further investigated by a response surface methodology.

3.2 Eutectic solvents or organics acids

L-dopa is an amino acid (pKa = 2.32), exhibiting the behavior of an alkaline compound under acidic conditions. Therefore, the choice of aqueous solutions based on organic acids comes in line with the concept of acid-base extraction, where a greater interaction between solvent-solute can occur and explain the high yields and selectivity observed. Since in a previous work,[24] we studied the use of eutectic systems to carry this extraction, Figure 3 reports a comparison between the performance the organic acid selected from the screening and the [Ch]Cl based eutectic solvents, allowing for an analysis of the effects caused by each specific component of the mixture. The results for the aqueous solutions of the two eutectic solvents ([Ch]Cl:CA (1:1 and 1:2), and for the aqueous solution of [Ch]Cl were previously reported by Benfica et al [24].

Levodopa moderate solubility in water leads to low extraction yields and poor selectivity (low purity) when water is used as solvent (3.6 ± 0.1 wt.%). The analysis of

the performance of the HBA component in water shows that choline chloride alone, at a concentration of 50 wt.%, is also not conducive to a significant or selective extraction of levodopa. The eutectic solvents based on CA present good yields and selectivity in the extraction of levodopa from mucuna seeds,[24] with the eutectic solvent [Ch]Cl:CA (1:2) at 50% in water showing a yield of (7.1± 0.4 wt.%). Surprisingly, the citric acid alone, at the same concentration, presents a yield of (8.0 ± 0.3 wt.%), higher than those observed for the eutectic systems. This is advantageous from the perspective of a process design since it allows the use a simpler solvent, that will be cheaper, and easier to manipulate and recover than an eutectic mixture.

It has been shown that acids such as citric acid and lactic acid have hydrotropic properties,[25,26] and in an acid environment they have the ability to interact with protonated low solubility drugs.[26] This explains the good performance observed in this work for these acids and the eutectic solvents based on them previously reported.[24]

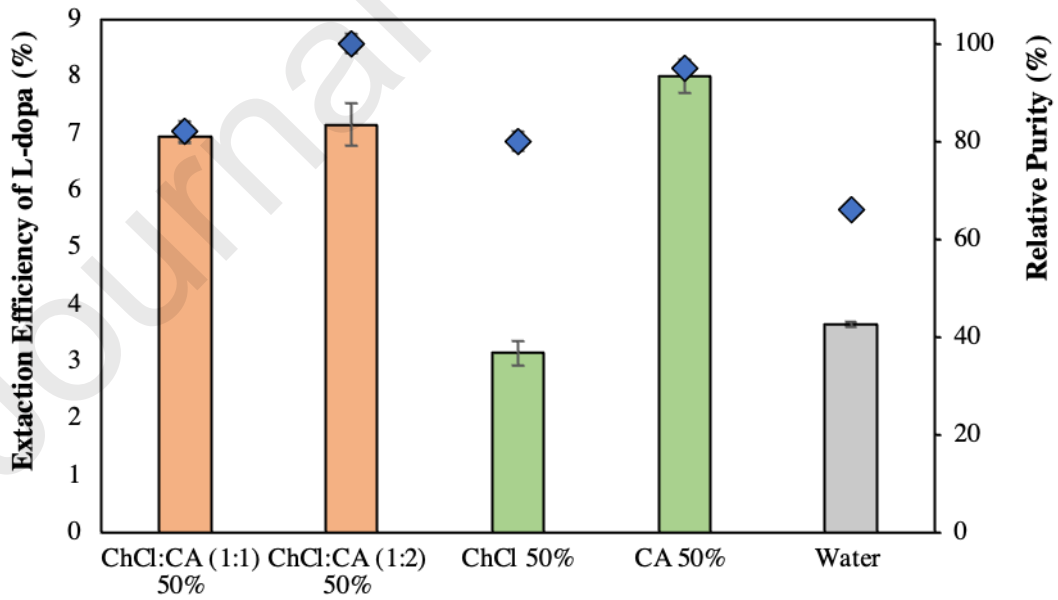

Figure 3. Comparation of aqueous solutions of eutectic solvents based in citric acid (■) and aqueous solutions of HBA and HBD (■) to extract levodopa, given by the extraction efficiency

(bars). Relative purity of levodopa (◆). Extraction performed at fixed conditions: T = 50°C, S:L ratio = 1:10, time = 90 min.

3.3 Optimization of Operational Conditions by Response Surface Methodology

To design a sustainable process, aqueous solutions of citric acid were selected as the most promising to extract levodopa, and were further used to optimize the extraction process conditions applying a response surface methodology. Here, the extraction temperature (T), the solid-liquid ratio (S:L) and citric acid concentration (C, wt.%) were the variables to optimize. The response surface methodology allows to identify possible mutual effects and interactions between the variables studied on the extraction efficiency. Figure 4 depicts the plots of the response surface (top) results showing the influence of each variable on the levodopa extraction efficiency, while the contour graph (bottom) shows the simultaneous interaction between the investigated variables.

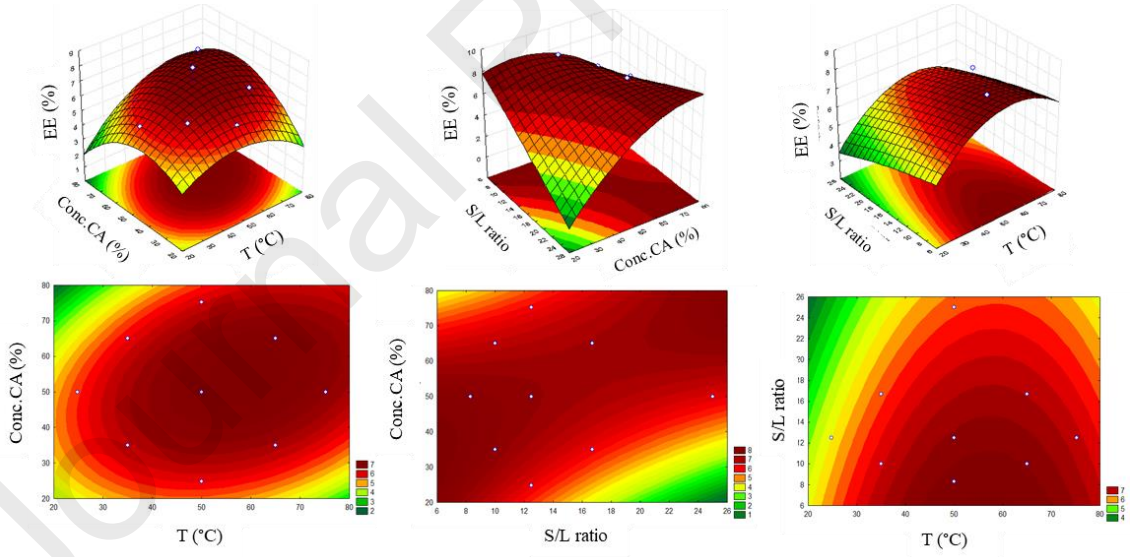

Figure 4. Surface graphs (up) and contour graphs (bottom) of the interactions of various factors in the extraction efficiency of levodopa. (i) temperature and concentration of citric acid; (ii) concentration of citric acid and solid/liquid ratio; and (iii) temperature and solid/liquid ratio.

The response surfaces reflect the co-relation between two independent variables and show the interactions of the extraction temperature (X_1), solid:liquid ratio (X_2) and citric acid concentration (X_3) in the *L*-dopa extraction efficiency. Analysis of variance (ANOVA) was used to determine the statistical significance of the variables and their interactions. The efficiency of extraction of *L*-dopa was used as the dependent variable in the definition of the predictive model represented by Eq. (3).

The model was adjusted with a confidence level of 95 % and can be considered as a highly predictive model. It was validated using the plot of the observed values versus predicted values. The average relative deviation between the observed and predicted values is 0.09 %, demonstrating a good description of the experimental results by the statistical model. The experimental design, the *L*-dopa extraction efficiencies and the correlation coefficients obtained, and all statistical analyses are given in the ESI (Tables S1 – S4 and Figure S1-S2).

$$EE_{levo}(\%) = 6.74 + 0.13\,X_1 - 0.001X_1{}^2 - 0.57X_2 + 0.02X_3 - 0.002X_3{}^2 + 0.001X_{13} + 0.009X_{23} \quad (3)$$

The extraction yield of L-dopa is directly linked to the concentration of solvent, S:L ratio and operating temperature of the extraction system. The statistical analysis and data shown in Figure 4 indicate that the linear and quadratic effects of temperature are significant in relation to the efficiency of L-dopa extraction. Higher temperatures decrease the viscosity of the solvent, decrease mass transfer constraints and increase the solubility of levodopa, thus favouring the extraction process.

The surface responses show an increase in the efficiency of extraction of levodopa with an increase in the solvent concentration up to a maximum of about 60 of wt.% citric acid, revealing that the extraction capacity of the solvent may be associated with its acidity and capacity hydrotropic in aqueous systems.

316 It is also noted that the S:L ratio is a significant parameter. In mass transfer principles,
317 the driving force within the solid is associated with the S:L ratio. The driving force
318 during mass transfer within the solid is higher when a higher solvent ratio is used,
319 resulting in an increased diffusion rate.[27] By increasing the S:L ratio, there is an
320 increase in the L-dopa extraction efficiency.

321 According to the RSM optimization, the optimal conditions for L-dopa extraction are 60
322 °C, a S:L ratio of 1:7 and a concentration of citric acid of 58 wt.%. At the optimized
323 conditions of the experimental design, a L-dopa extraction efficiency of 9.2 ± 0.6 wt.%
324 was obtained. This value is competitive with the best value obtained with volatile
325 organic solvents, namely methanol (9.5 wt.%) shown in Figure 2 and those reported in
326 the literature.[10] Furthremore, data from literature using mixtures of ethanol:water in
327 presence of ascorbic acid, chloroform in alkaline media and mixtures of acetonitrile and
328 methanol under ultrasounds reported extraction efficiencies of levodopa in the range
329 between 1.8 and 7.6 wt.%. [11,14]

330 The alternative solvent approach proposed by Benfica et al. [24] using aqueous
331 solutions of eutectic solvents based on lactic acid to extract levodopa achieve significant
332 yield results of (9.9 ± 1.0 wt.%). Although good yields and purity are obtained, the use
333 of an alternative method even simpler and easy to separate proposed here in this work
334 for aqueous citric acid solutions (9.2 ± 0.6 wt.%) is even more attractive. In mild
335 conditions and temperatures, citric acid is proposed here as a competitive and more
336 effective solvent for extracting levodopa from biomass, allowing the replacement of the
337 commonly used volatile organic solvents.

338 *3.4 Recovery of L-dopa and recycle of solvent*

339 Regardless of the efficiency of the extraction process, the solvent recovery and its
340 recyclability are two conditions to develop a sustainable process. Accordingly, in this
341 work, it was attempted the recovery of L-dopa from the citric acid aqueous solution and
342 the solvent reuse in new cycles of extraction with fresh biomass. To accomplish this
343 goal, the extraction steps were performed at the optimized conditions (T = 60 ° C, ratio
344 S:L 1:7 and citric acid concentration of 58 wt.%). Figure 5 depicts the extraction
345 efficiency, relative purity and recovery yield of levodopa along the cycles using the
346 recycled solvent. Approximately 9.2 to 9.8 wt.% of L-dopa was extracted at the
347 optimized conditions along the several cycles, and purities ranging between 80 and 84%
348 were obtained. These results indicate that the reused solvent does not compromise the
349 extraction efficiency and selectivity towards levodopa. About 84% of L-dopa was
350 recovered by solid-phase extraction after the first cycle. However, a decrease to 80%
351 was observed in the following cycles, which may be due to the column separation
352 performance. The integrity of the recycled solvent was confirmed by ^{1}H and ^{13}C NMR,
353 as shown in the ESI (Figure S3), revealing that no degradation of the solvent occurs. ^{1}H
354 NMR was also used to confirm the integrity and purity of the recovered levodopa after
355 the extraction and recovery step (Figure 6).

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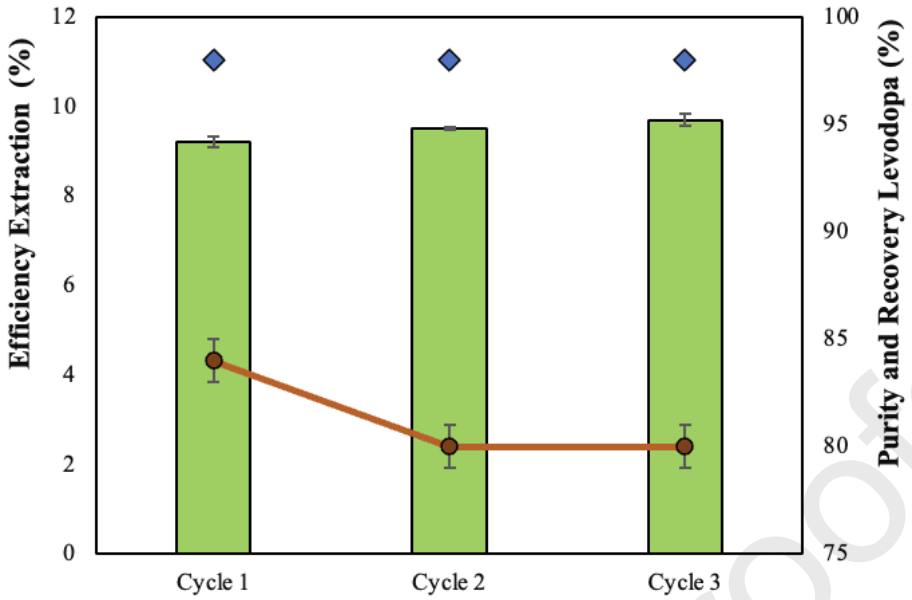


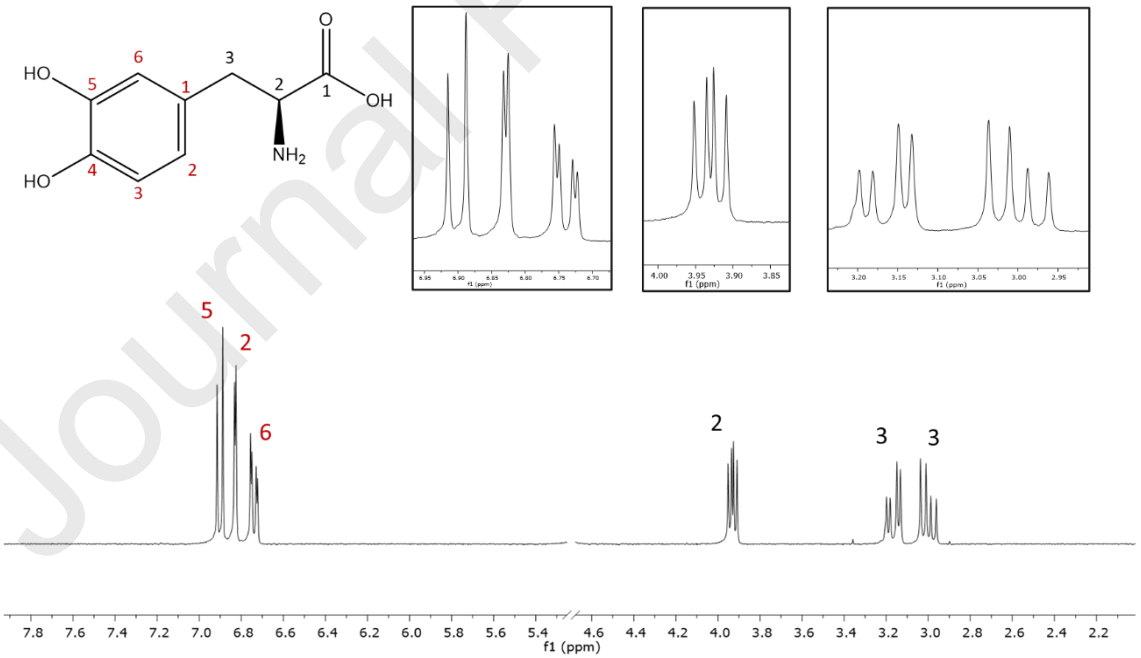
357

358 **Figure 5.** Efficiency Extraction (■), Recovery (●) and Purity (◆) of levodopa using

359 aqueous solutions of citric acid at the optimized conditions and a cation exchange

360 column for levodopa recovery, along 3 cycles of extraction involving the solvent reuse.

361



362

363 **Figure 6.** ^{1}H NMR spectra of the recovered levodopa from aqueous solutions of citric

364 acid.

365

4. Conclusions

M. pruriens seeds are a good source of levodopa, with potential application in the pharmaceutical industry to treat neurodegenerative disorders. Although its high relevance, the extraction of levodopa from biomes is usually carried out with volatile organic solvents. In this work, it was shown that aqueous solutions of carboxylic acids, and in particular of citric acid which is already used in the food and pharmaceutical industries, allows to obtain and effective and selective extraction of L-dopa from *M. pruriens* seeds. By a response surface methodology, the optimal operational conditions identified to improve the extraction efficiency were 60 ° C, S:L of 1:7, acid concentration of 58 wt.%) and 90 minutes of extraction, leading to and extraction efficiency of L-dopa of 9.2 wt.%. This value is similar to the extraction efficiency obtained with pure methanol, with the additional advantage of being a more selective solvent. The aqueous solution can be recovered and reused, at least for 3 times, with no significant losses in extraction and selectivity performance. Levodopa can be recovered from the acidic aqueous solutions by solid-phase extraction, with recovery yields ranging between 80-84%. The high purity of the recovered levodopa was confirmed by NMR. Overall, the proposed solvent and process is more sustainable than other processes currently used and disclosed for the extraction of levodopa, making use of an aqueous solutions comprising a natural, low-cost and readily available organic acid.

Acknowledgment

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References

[1] H. Pulikkalpura, R. Kurup, P.J. Mathew, S. Baby, Levodopa in Mucuna pruriens and its degradation, Sci. Rep. 5 (2015) 2–10. https://doi.org/10.1038/srep11078.

[2] J. Aldred, J. Nutt, Levodopa, J. Med. Chem. 7 (2010) 132–137.

[3] S. Duty, P. Jenner, Animal models of Parkinson ' s disease : a source of novel treatments and clues to the cause of the, (2011) 1357–1391. https://doi.org/10.1111/j.1476-5381.2011.01426.x.

[4] S.A. Jagmag, N. Tripathi, S.D. Shukla, S. Maiti, Evaluation of Models of Parkinson ' s Disease, 9 (2016). https://doi.org/10.3389/fnins.2015.00503.

[5] P. Rev, L. Sathiyanarayanan, S. Arulmozhi, Mucuna pruriens Linn . - A Comprehensive Review, Pharmacogn. Rev. 1 (2007) 157–162.

[6] J.H. Kordower, C.G. Goetz, The first miracle in neurodegenerative disease : The discovery of oral levodopa, 50 (1999) 377–378. https://doi.org/10.1016/S0361-9230(99)00112-4.

[7] H.F. Martins, D.P. Pinto, V. de A. Nascimento, M.A.S. Marques, F.C. Amendoeira, Determination of Levodopa in Human Plasma By High Performance Liquid Chromatography–Tandem Mass Spectrometry (Hplc–Ms/Ms): Application To a Bioequivalence Study, Quim. Nova. 36 (2013) 171–176. https://doi.org/10.1590/S0100-40422013000100028.

[8] O. Tucha, L. Mecklinger, J. Thome, A. Reiter, G.L. Alders, Kinematic analysis of dopaminergic effects on skilled handwriting movements in Parkinson ' s disease, (2006) 609–623. https://doi.org/10.1007/s00702-005-0346-9.

[9] M. Akhbari, S. Hamedi, Z. sadat Aghamiri, Optimization of total phenol and anthocyanin extraction from the peels of eggplant (Solanum melongena L.) and biological activity of the extracts, J. Food Meas. Charact. 13 (2019) 3183–3197. https://doi.org/10.1007/s11694-019-00241-1.

[10] C.A. Anosike, O.N. Igboegwu, O.F.C. Nwodo, Antioxidant properties and membrane stabilization effects of methanol extract of Mucuna pruriens leaves on normal and sickle erythrocytes, J. Tradit. Complement. Med. (2018) 1–7. https://doi.org/10.1016/j.jtcme.2017.08.002.

[11] L. Misra, H. Wagner, Extraction of bioactive principles from Mucuna pruriens seeds, Indian J. Biochem. Biophys. 44 (2007) 56–60.

[12] F. Chemat, M. Abert Vian, A.-S. Fabiano-Tixier, M. Nutrizio, A. Režek Jambrak, P.E.S. Munekata, J.M. Lorenzo, F.J. Barba, A. Binello, G. Cravotto, A review of sustainable and intensified techniques for extraction of food and natural products, Green Chem. (2020). https://doi.org/10.1039/c9gc03878g.

[13] J. Płotka-Wasylka, J. Namieśnik, Green Chemistry and Sustainable Technology Green Analytical Chemistry, Springer Singapore, 2019. https://doi.org/10.1007/978-981-13-9105-7.

[14] M. Rane, S. Suryawanshi, R. Patil, C. Aware, R. Jadhav, S. Gaikwad, P. Singh, S. Yadav, V. Bapat, R. Gurav, J. Jadhav, Exploring the proximate composition , antioxidant , anti-Parkinson's and anti-in fl ammatory potential of two neglected and underutilized Mucuna species from India, South African J. Bot. 124 (2019)

304–310. https://doi.org/10.1016/j.sajb.2019.04.030.

[15] F. Chemat, M. Abert-vian, A.S. Fabiano-tixier, J. Strube, L. Uhlenbrock, V. Gunjevic, G. Cravotto, Trends in Analytical Chemistry Green extraction of natural products . Origins , current status , and future challenges, Trends Anal. Chem. 118 (2019) 248–263. https://doi.org/10.1016/j.trac.2019.05.037.

[16] R.N. Cavalcanti, T. Forster-Carneiro, M.T.M.S. Gomes, M.A. Rostagno, J.M. Prado, M.A.A. Meireles, Uses and applications of extracts from natural sources, 2013. https://doi.org/10.1039/9781849737579-00001.

[17] G.S. Dhillon, S.K. Brar, Recent Advances in Citric Acid Bio-production and Recovery, 2009 (2011) 505–529. https://doi.org/10.1007/s11947-010-0399-0.

[18] A.R. Angumeenal, D. Venkappayya, An overview of citric acid production, LWT - Food Sci. Technol. 50 (2013) 367–370. https://doi.org/10.1016/j.lwt.2012.05.016.

[19] R. Ciriminna, F. Meneguzzo, R. Delisi, M. Pagliaro, Citric acid : emerging applications of key biotechnology industrial product, Chem. Cent. J. (2017) 1–9. https://doi.org/10.1186/s13065-017-0251-y.

[20] E.P. Serjeant, B. Dempsey, I.U. of P. and A. Chemistry., C. on E. Data., Ionisation constants of organic acids in aqueous solution, Pergamon Press, Oxford; New York, 1979.

[21] S.R. Palit, Solvent action and “co-solvency”: A lecture demonstration, J. Chem. Educ. 23 (1946) 182. https://doi.org/10.1021/ed023p182.

[22] B. Soares, A.J.D. Silvestre, P.C. Rodrigues Pinto, C.S.R. Freire, J.A.P. Coutinho, Hydrotropy and Cosolvency in Lignin Solubilization with Deep Eutectic

461 Solvents, ACS Sustain. Chem. Eng. (2019).
462 https://doi.org/10.1021/acssuschemeng.9b02109.

463 [23] M. Jacotet-Navarro, M. Laguerre, A.S. Fabiano-Tixier, M. Tenon, N. Feuillère,
464 A. Bily, F. Chemat, What is the best ethanol-water ratio for the extraction of
465 antioxidants from rosemary? Impact of the solvent on yield, composition, and
466 activity of the extracts, Electrophoresis. 39 (2018) 1946–1956.
467 https://doi.org/10.1002/elps.201700397.

468 [24] J. Benfica, J. S. Miranda, E. S. Morais, M. G. Freire, J. A.P. Coutinho, R. de
469 Cássia Superbi de Sousa, Enhanced extraction of levodopa from Mucuna pruriens
470 seeds using aqueous solutions of eutectic solvents, ACS Sustain. Chem. &
471 Eng. 8 (2020) 6682–6689. https://doi.org/10.1021/acssuschemeng.0c00196.

472 [25] E. Baka, J.E.A. Comer, K. Takács-Novák, Study of equilibrium solubility
473 measurement by saturation shake-flask method using hydrochlorothiazide as
474 model compound, J. Pharm. Biomed. Anal. 46 (2008) 335–341.
475 https://doi.org/10.1016/j.jpba.2007.10.030.

476 [26] E. Shoghi, E. Fuguet, E. Bosch, C. Ràfols, Solubility – pH profiles of some
477 acidic , basic and amphoteric drugs, Eur. J. Pharm. Sci. 48 (2013) 291–300.
478 https://doi.org/10.1016/j.ejps.2012.10.028.

479 [27] J.E. Cacace, G. Mazza, Mass transfer process during extraction of phenolic
480 compounds from milled berries, J. Food Eng. 59 (2003) 379–389.
481 https://doi.org/10.1016/S0260-8774(02)00497-1.

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Supporting Information Captions

Aqueous Solutions of Organic Acids as Effective Solvents for Levodopa Extraction from *Mucuna pruriens* Seeds

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Table: 5

Table S1. Compound Descriptions, Molecular formula, CAS Number, Purity and Supplier of the ES Components Investigated.

Compounds	Molecular	CAS number	Purity/wt %	Supplier
Acetic acid (AA)	$C_2H_4O_2$	64-19-7	99.9	Honeywell
Citric acid (CA)	$C_6H_8O_7$	77-92-9	99.5	Panreac
Glycolic acid (GA)	$C_2H_4O_3$	79-14-1	99.0	Acros Organics
Lactic acid (LA)	$C_3H_6O_3$	10326-41-7	90.0	Sigma Aldrich
Propionic acid (PA)	$C_3H_6O_2$	79-09-4	99.0	Acros Organics
Ethylene glycol (EG)	$C_2H_6O_2$	107-21-1	99.5	Sigma Aldrich
Glycerol (G)	$C_3H_8O_3$	56-81-5	99.8	Fisher Chemical
Hexane	C_6H_{14}	110-54-3	99.0	Carlo Erba
Methanol	CH_4O	67-56-1	HPLC	Fisher Scientific

Table S2. Levels of process factors in experimental design.

Parameters	Levels				
	-1.682	**-1**	**0**	**1**	**1.682**
Temperature (°C)	24.8	35	50	65	75.2
Solid-liquid ratio	25.0	16.7	12.5	10	8.3
ES Concentration (wt.%)	24.8	35	50	65	75.2

523 **Table S3.** Results of response surface methodology for the extraction of levodopa from

524 *Mucuna pruriens* seeds using aqueous solution of CA.

Assay	Coded values			Conditions			Response
	X_1	X_2	X_3	T (°C)	S:L	CA (%)	EE_{levo} (Y)
1	-1	-1	-1	35	16.7	35	5.81
2	-1	-1	1	35	16.7	65	5.75
3	-1	1	-1	35	10	35	6.11
4	-1	1	1	35	10	65	6.15
5	1	-1	-1	65	16.7	35	5.88
6	1	-1	1	65	16.7	65	5.87
7	1	1	-1	65	10	35	6.07
8	1	1	1	65	10	65	6.61
9	-1.682	0	0	24.8	12.5	50	6.11
10	1.682	0	0	75.2	12.5	50	5.99
11	0	-1.682	0	50	25	50	5.46
12	0	1.682	0	50	8.3	50	6.51
13	0	0	-1.682	50	12.5	24.8	6.02
14	0	0	1.682	50	12.5	75.2	5.94
15	0	0	0	50	12.5	50	5.86
16	0	0	0	50	12.5	50	5.97
17	0	0	0	50	12.5	50	5.95
18	0	0	0	50	12.5	50	5.93
19	0	0	0	50	12.5	50	5.97

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529 **Table S4.** Estimated coefficients obtained from the polynomial model and statistical
530 criteria of extraction using aqueous solutions of CA[.

Parameters	Coefficients	Standard deviation	t-student (4)	P-value	Confidence Limit -95%	Confidence Limit +95%
Interception	6.746	0.353	19.07	0.0000	5.76	7.72
Temperature	0.139	0.006	20.38	0.0000	0.12	0.15
Temperature ^{2}	-0.001	0.000	-31.09	0.0000	-0.00	-0.00
Solid-Liquid ratio	-0.571	0.016	-34.02	0.0000	-0.61	-0.52
Concentration	0.023	0.008	2.82	0.0476	0.00	0.04
Concentration ^{2}	-0.002	0.000	-37.18	0.0000	-0.00	-0.00
Temperature x Concentration	0.001	0.000	16.99	0.0000	0.00	-0.00
Solid-liquid ratio x concentration	0.009	0.000	29.40	0.0000	0.00	0.01

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532 **Table S5.** ANOVA data for the extraction of levodopa obtained from RSM design
533 using aqueous solutions of CA

Factors	Sum of squares	Degree of freedom	Mean Square	F-value	P-value
Temperature	2.18	1	2.18	963.49	0.000006
Temperature ^{2}	2.19	1	2.19	966.88	0.000006
Solid-Liquid ratio	1.86	1	1.86	819.58	0000008
Concentration	0.07	1	0.07	32.20	0.004
Concentration ^{2}	3.13	1	3.13	1382.40	0.000003
Temperature x Concentration	0.65	1	0.65	288.77	0.00007
Solid-liquid ratio x concentration	1.96	1	1.96	864.61	0.000007
Lack or Fit	0.90	7	0.12		
Pure Error	0.009	4	0.002		-

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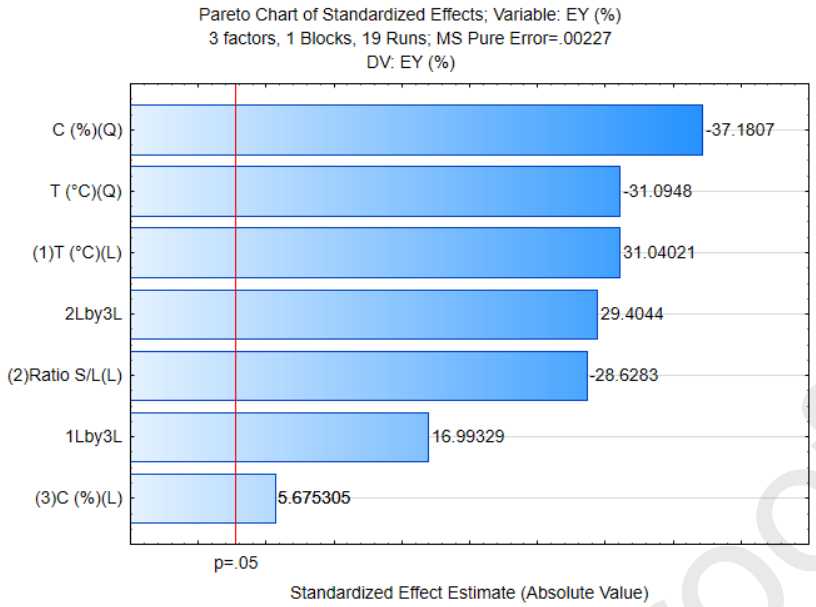


Figure S1. *Pareto diagram for the effects of process parameters on the extraction of levodopa from Mucuna pruriens seeds using aqueous solutions of CA.*

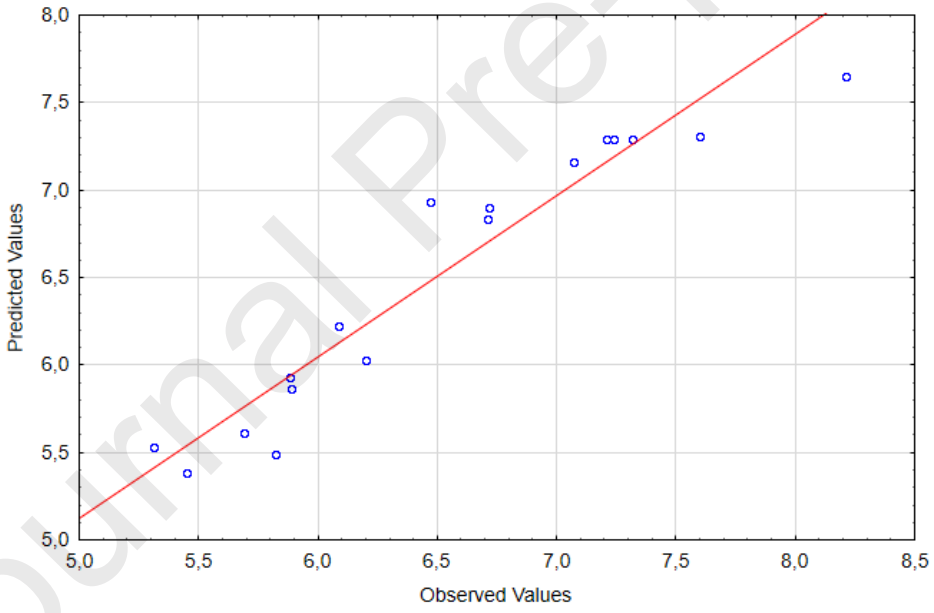
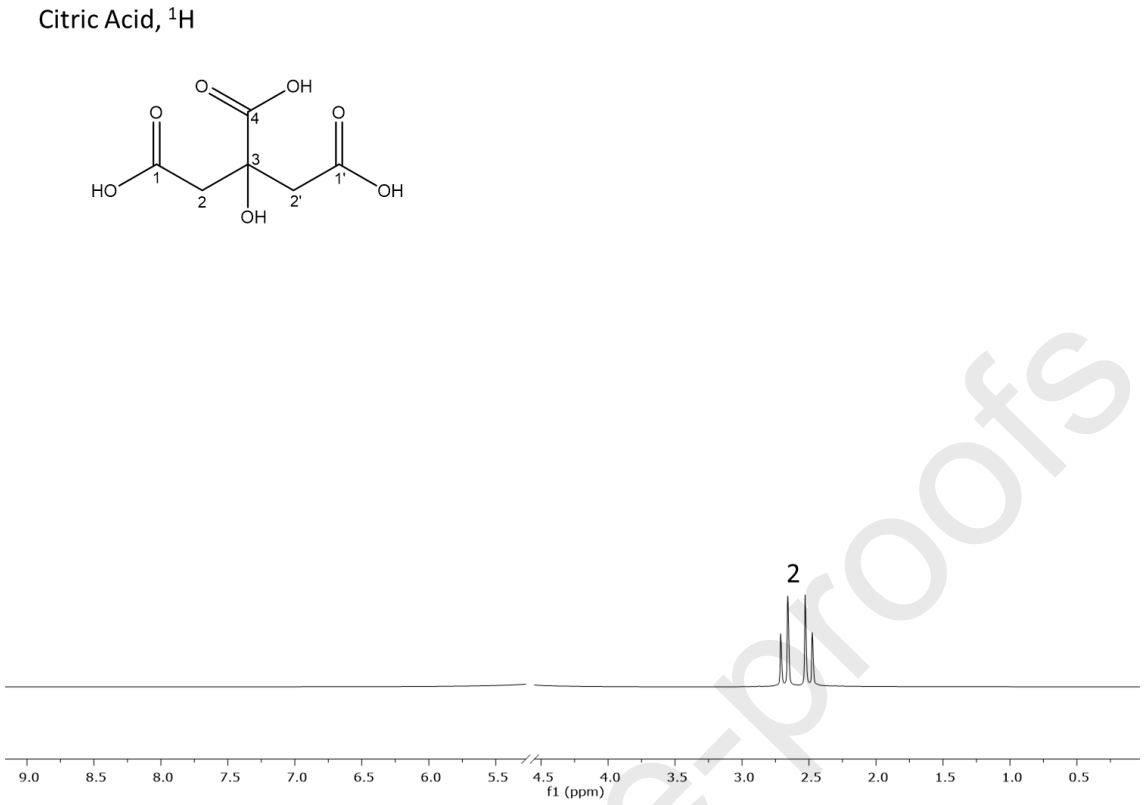


Figure S2. *Predicted and observed values obtained from RSM design using aqueous solutions of CA.*



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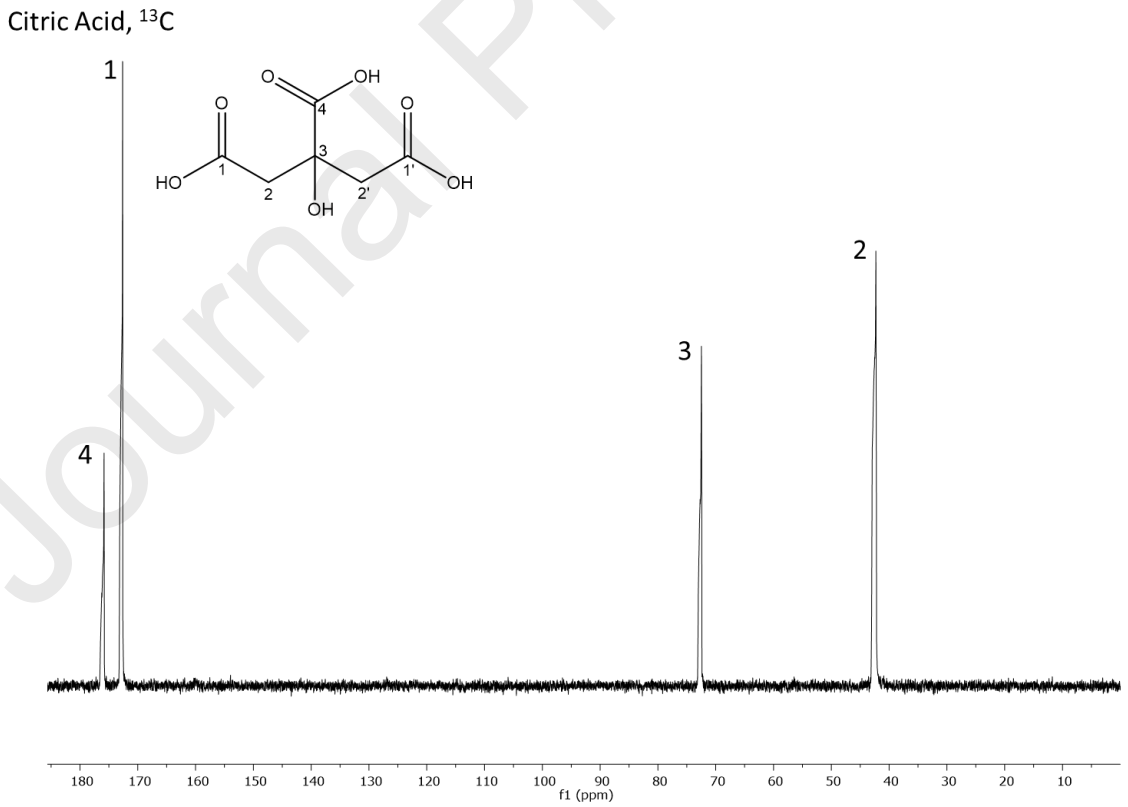

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553 **Figure S3.** *Results of NMR 1H and 13C from aqueous solutions of CA.*

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

AUTHOR CONTRIBUTIONS

Jordana Benfica: Investigation; Methodology; Writing - Original Draft;

Eduarda S. Morais: Visualization; Writing - Review & Editing;

Julia S. Miranda: Writing - Review & Editing

Mara G. Freire: Conceptualization; Writing - Review & Editing ; Supervision

Rita de Cássia S. de Sousa: Writing - Review & Editing ; Supervision

João A. P. Coutinho: Conceptualization; Writing - Review & Editing; Supervision