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ORIGINAL PAPER

Chemical profiling and characterization of polar extracts from *Dipteryx odorata* seeds using capillary electrophoresis-based protocols

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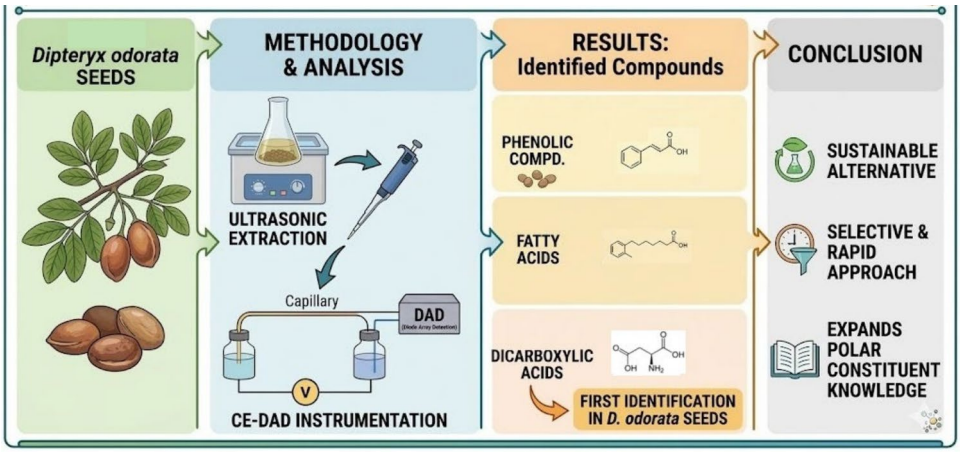
Abstract

Chemical profiling of secondary metabolites in polar extracts of *Dipteryx odorata* seeds was carried out using capillary electrophoresis (CE) protocols. *D. odorata* seeds are widely used in gourmet cuisine, highlighting the need for rapid and reliable analytical methods to assess their chemical profile and quantify bioactive compounds for the quality control of both raw seeds and commercial extracts. In this study, polar extracts obtained from commercial *Dipteryx odorata* seed samples were analyzed using optimized CE-based protocols. Direct UV detection was applied for the determination of phenolic compounds (including coumarins, flavonoids, and phenolic acids) and alkaloids, whereas indirect UV detection was used to quantify fatty acids and carboxylic acids. Among the phenolic compounds, *o*-coumaric acid (0.02% w/w) was identified and quantified. The major fatty acids identified were oleic (C18:1), linoleic (C18:2), palmitic (C16:0), and stearic (C18:0) acids, with concentrations of 21.69%, 8.43%, 2.73%, and 6.27% (w/w), respectively. Carboxylic acid analysis revealed levels of 0.16%, 0.41%, and 0.22% (w/w) for aspartic, malic, and glutamic acids, respectively. To the best of our knowledge, this is the first report on the use of CE to perform chemical profiling of *D. odorata* seeds. CE-based protocols demonstrated high specificity and selectivity, allowing the direct injection of crude extracts without the need for pretreatment and contributing to a fast, low-solvent method for quality control of *D. odorata* seeds.

Graphical Abstract



Introduction

Dipteryx odorata seeds (or tonka beans) come from a tropical tree native to northern South America, particularly the Brazilian states of Amazonas and Pará [1]. These seeds are widely utilized in perfumery, natural cosmetics, and traditional medicine. In gourmet cuisine, chefs use the seeds as

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a flavoring agent in desserts, chocolates, and craft beverages, as a substitute for vanilla. The seeds are characterized by a high coumarin content (1–10% w/w), responsible not only for their distinctive aroma, reminiscent of vanilla and almond, but also for reported biological activities [2, 3]. In addition to coumarin, other secondary metabolites have been identified, including phenolic acids (e.g., o-coumaric acid), coumarins (e.g., umbelliferone), flavonoids, fatty acids, and other bioactive compounds [4–10].

The volatile fraction of the seeds has been extensively characterized [11, 12], whereas studies addressing polar seed extracts remain scarce. Previous investigations have predominantly applied chromatographic techniques such as GC–MS, HPLC–DAD, HPLC–MS, and GC–FID. While these approaches provide detailed chemical characterization of bioactive compounds, they typically involve labor-intensive sample preparation and derivatization, particularly for polar analytes [6, 8, 13, 14]. Therefore, the development of rapid analytical methods allowing direct injection with minimal sample preparation, eliminating fractionation steps, and supporting authentication strategies is highly desirable.

Capillary electrophoresis (CE) is a promising alternative technique for chemical and phytochemical profiling of complex matrices [11, 12]. Its high efficiency and selectivity, low solvent consumption, a rapid analysis time and the possibility of direct analysis without make it an attractive alternative to chromatographic methods. Systematic procedures for investigating secondary metabolites in herbal extracts were previously developed using CE, and are referred as CE-based protocols [13]. This approach involves adjusting the appropriate pH conditions and selecting suitable electrolytes, with or without additives, to achieve the ionization of specific phytochemical classes. Both CE modes, free-solution capillary electrophoresis (FSCE), used for the separation of ionic compounds, and micellar electrokinetic chromatography (MEKC), suitable for the separation of both neutral and ionic compounds can be applied. The wavelength must be carefully selected to detect the target compounds of interest while minimizing interference from other compounds present in the extract. Crude extracts are injected without prior purification (clean-up steps), under conditions that allow specific ionization of the phytochemical groups of interest, ensuring selectivity.

Santos et al. applied CE-based protocols to investigate the compounds responsible for the allelopathic activity of organic extracts from leaves and roots of *Canavalia ensiformis* [14, 15]. The authors concluded that these protocols were valuable for obtaining a preliminary characterization of the phytochemical groups present in crude extracts, which could guide bioassay-directed studies. Ribeiro et al. used CE protocols to identify the main classes of secondary metabolites in crude extracts and fractions from roots and shoots of the forage grass *Urochloa humidicola* (Rendle) Morrone & Zuloaga. Their results indicated that polar compounds, particularly flavonoids and phenolic acids, were associated with the allelopathic activity observed in the evaluated plants [16]. Similarly, CE protocols were used also to screen organic and aqueous extracts from *Casearia ensiformis* leaves, enabling the identification and quantification of rutin and scopoletin, along with other secondary metabolites [17].

Coumarin is the main metabolite found in *D. odorata* seeds. Due to its hepatotoxicity and regulatory relevance, its determination is critical for quality control purposes (ref). In a previous study, a CE-based protocol using MEKC was developed for the separation of coumarins and coumaric acids in crude polar extracts and fractions obtained from commercial *D. odorata* seed samples [10]. In addition to coumarin, *D. odorata* seeds contain a variety of primary and secondary metabolites that may contribute to their chemical characterization and authenticity assessment. Fatty acids are relevant constituents that can serve as indicators of lipid composition and potential quality attributes. Phenolic compounds also play an important role as potential chemical markers due to their structural diversity and occurrence in plant matrices.

Unlike our previous targeted CE study, which focused on the quantification of two main compounds, the present work expands the analytical scope toward broader profiling of secondary metabolites present in *D odorata* seeds, using CE-based protocols in FSCE mode. This approach provides a rapid, selective, and environmentally friendly method for the chemical characterization of this species. To the best of our knowledge, the use of FSCE for the chemical characterization of polar extracts from *D. odorata* seeds has not yet been reported.

Materials and methods

Chemicals and reagents

Methanol (MeOH), hexane (HX), dichloromethane (DCM), acetonitrile (ACN), ethanol (EtOH), sodium hydroxide (NaOH), ethyl acetate (EtOAc), cetyltrimethylammonium bromide (CTAB), 3.5 dinitrobenzoic acid (3.5 DNB), sodium dodecyl sulphate (SDS), dodecylbenzenesulfonate (SDBS) and 1-octanol were purchased from Synth (Rio de Janeiro, Brazil). Sodium phosphate ($NaHPO_4.2H_20$ and $Na_2HPO_4.2H_20$), sodium tetraborate ($Na_2B_4O_7.10H_2O$), ammonium formate (NH_4HCO_2) and dimethyl sulfoxide (DMSO) were obtained from Merck (Rio de Janeiro, Brazil). Polyoxyethylene 23 lauryl ether (BRij35) and all of the standards of secondary metabolites were obtained from Sigma-Aldrich (St. Louis, USA).

Sample preparation

Commercial samples of *D. odorata* seeds were purchased from Flordejambu (São Paulo, Brazil). As the material was obtained through wildcrafting, and marketed without batch identification. Botanical identification was performed by the authors based on macroscopic characteristics (size, surface texture, color, and odor) and comparison with published photographic records [18]. The voucher specimen must be deposited in the Herbarium of the Botanical Institute of São Paulo, Brazil. Extraction was carried out according to [10]. Powdered seeds (5.00 g) were extracted with 100 mL of MeOH in an ultrasonic bath (Cristofoli Equipamentos, Paraná, Brazil) for 15 min at room temperature. The extract was filtered (qualitative filter paper) and evaporated to dryness under reduced pressure. The residue was reconstituted in 1 mL of MeOH, evaporated to dryness under a nitrogen stream, weighed, and re-dissolved in 1 mL of MeOH:H_2O (1:1, v/v) prior to CE analysis. Samples were filtered through a 0.22 µm membrane filter (Millex, Millipore, USA) directly into injection vials. All extractions were performed in triplicate. For fatty acid determination, a saponification step was necessary before injection. The saponification procedure was adapted from a previously validated method reported in the literature originally developed for efficient hydrolysis and recovery of fatty acids [19]. The saponification step was carried out in a hot water bath (75–80 °C) for 25 min, using 200 mg of ground seed and 2 mL of a methanolic NaOH solution (0.5 mol L^{-1}) under reflux. Before injection, samples were diluted in methanol 1:10.

Standards and stock buffer solutions preparation

Reference standards of phenolic acids, flavonoids, and alkaloids were prepared as individual mixtures in ethanol at the working concentrations listed in Table 1. Carboxylic acid standards were prepared in water, whereas fatty acid standards were initially dissolved in methanol and subsequently diluted with water to obtain the appropriate working concentrations. Stock electrolyte solutions were prepared in deionized water (Milli-Q system, Millipore, Bedford, MA, USA) at the following concentrations: sodium phosphate and sodium tetraborate (100 mmol L^{-1}), SDS (100 mmol L^{-1}), CTAB and SDBS (10 mmol L^{-1}), 3.5-DNB and Brij 35 (50 mmol L^{-1}). Working electrolyte concentrations are summarized in Table 1.

CE instrumentation

Separations were carried out on a PA 800 Plus capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a diode-array UV detector. Analyses were performed using a fused-silica capillary with a polyimide outer coating (Polymicro, Phoenix, AZ, USA) and an internal diameter of 75 µm. The effective and total capillary lengths, as well as conditioning procedures, are summarized in Table 2.

Table 1 Electrolyte concentration and optimized conditions used in each CE protocol

Protocols	Standards and concentration	Electrolyte	Voltage (kV)	ƛ (nm)
Protocol I	histamine, cinchonidine, quinine, scopolamine, atropine (20 mg L^{-1})	Sodium phosphate 30 mmol L^{-1} (pH 2.5)	20	220
Protocol II	rutin, naringenin, scopoletin, umbelifferone kaempferol, quercetin, (20 mg L^{-1})	Sodium tetraborate 40 mmol L^{-1} (pH 9.0)	22	350
Protocol III	ferulic, vanillic, o-coumaric, caffeic, 4-hydroxybenzoic, chlorogenic acid (20 mg L^{-1})	Sodium phosphate 20 mmol L^{-1} (pH 6.9)	22	214
Protocol IV	malic, malonic, glutamic, aspartic, lactic acids (25 mg L^{-1})	DNB 10 mmol L^{-1}/ CTAB 0.2 mmol L^{-1} (pH 3.6)	−15	254
Protocol V	oleic, linoleic, palmitic, stearic acids (250 mg L^{-1})	Sodium phosphate 15 mmol L^{-1} (pH 6.8)/SDBS 4 mmol L^{-1}/ BRIJ 35 10 mmol L^{-1}/1-octanol 2%/ ACN 45%	−20	224

Injection by pressure (0,3 psi): Protocols I, II and III – 10 s; Protocol IV – 6 s; Protocol V – 3 s. Temperature: 25 °C. Legend: DNB—3.5 dinitrobenzoic acid; CTAB – cetyltrimethylammonium bromide; SDBS – dodecylbenzenesulfonate; BRIJ35—polyoxyethylene 23 lauryl ether; ACN—acetonitrile

Identification and quantification of secondary metabolites and figure of merit

Peak identification was achieved based on standard addition (spiking), by migration time and UV spectral matching with standards. Dicarboxylic acids were confirmed also by Mass

Table 2 Screening experiments: capillary dimensions and conditioning

	Protocols I, II, III and IV	Protocol V
Total length	49 cm	60 cm
Effective length	39 cm	50 cm
Initial conditioning	20 min NaOH 1 mol L^{-1}/20 min H_2O/25 min electrolyte	15 min NaOH 0.1 mol L^{-1}/15 min H_2O/10 min electrolyte
Conditioning between runs	1 min NaOH 1 mol L^{-1}/1 min H_2O/3 min electrolyte	2.5 min NaOH 0.1 mol L^{-1}/1 min H_2O/2.5 min electrolyte

Spectrometer analysis and fatty acids by Nuclear Magnetic Resonance. Quantification was performed by external calibration using five concentration levels each. The analysis was performed in triplicate. The figures of merit were evaluated for protocols III, IV, and V, in which compounds were identified and included linearity, limit of detection (LOD) and limit of quantification (LOQ), precision and migration time repeatability. Linearity was assessed for each protocol using calibration curves at six concentration levels, based on the coefficient of determination (R^2). The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the standard deviation of the response (σ) and the slope of the calibration curve (S), using the equations LOD = 3.3σ/S and LOQ = 10σ/S, according to ICH guidelines [20]. Precision was evaluated at three concentration levels (low, medium, and high) and expressed as relative standard deviation (RSD). Repeatability was assessed by six consecutive injections of the target analyte in each protocol, and expressed as coefficient of variation (CV).

Mass spectrometer (MS) analysis and RMN analysis

Mass spectrometric analyses were performed using a CE–ESI–MS system. Injections were carried out on an Agilent 7100 CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a fused-silica capillary (50 µm i.d., 55 cm total length). The running electrolyte consisted of 30 mmol L^{-1} ammonium formate (pH 6.5). Separation conditions were as follows: applied voltage of 25 kV, capillary temperature of 20 °C, and hydrodynamic injection for 15 s at 100 mbar (sample diluted 1:5). The CE system was coupled to an Agilent 6430 Triple Quadrupole LC/MS instrument with an electrospray ionization (ESI) source operating in both positive and negative ion modes. The sheath liquid consisted of 5 mmol L^{-1} ammonium formate in MeOH:H_2O (50:50, v/v), pH 6.5, delivered at 6.0 µL min^{-1}. The ESI parameters were: capillary voltage, 4000 V; drying gas (N_2) flow, 3.0 L min^{-1}; drying gas temperature, 220 °C; nebulizer pressure, 7 psi. Selected ion monitoring (SIM) was performed in the m/z range 87–400 with a fragmentor voltage of 16 V and a dwell time of 50 ms. ^{1}H NMR spectra were recorded on an Ultrashield 300 Bruker Avance III spectrometer operating at 300 MHz (^{1}H). Samples were dissolved in DMSO-d_6 prior to analysis.

Results and discussion

CE protocols are based both on the choice of the appropriate pH and background electrolyte, which allows the ionization of the specific phytochemical class. Direct UV detection conditions are optimized according to the spectral characteristics of each analyte class to ensure selectivity within the complex extract matrix. Alkaloids, flavonoids and phenolic acids present direct UV absorption. Indirect UV detection is applied for compounds with low UV–Vis absorbance. In this approach, a chromophore reagent is added to the background electrolyte, and analytes are detected as transient decreases in the chromophore signal as they pass through the detector [21]. Under these conditions, carboxylic and fatty acids were determined. CE protocols used in this study are summarized in Fig. 1.

Sonication-assisted extraction using methanol as the solvent was selected based on our previous study [22, 23]. Methanol was chosen due to its well-established ability to efficiently extract and solubilize a wide range of polar and moderately polar metabolites, making it an appropriate model solvent for such extracts.

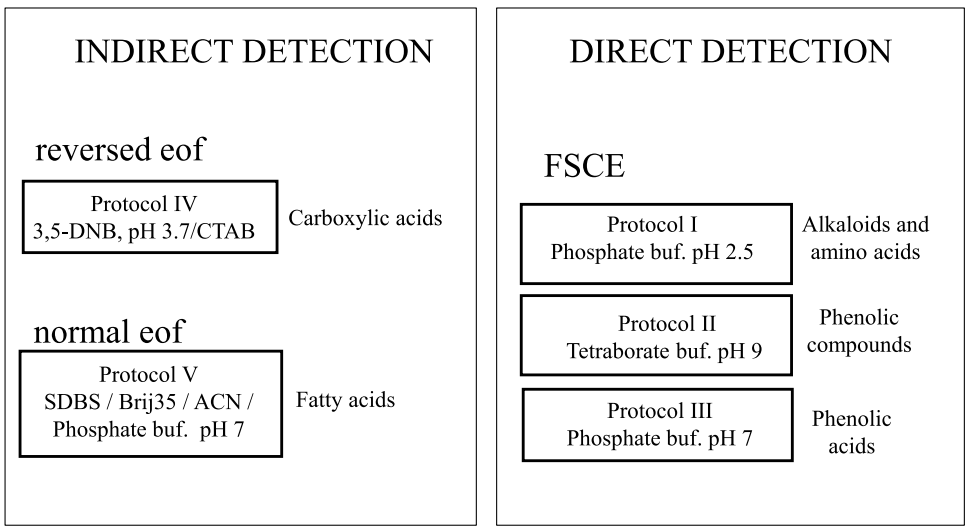

Fig. 1 CE protocols scheme

CE protocols using direct UV absorbance detection

Protocol I – investigation of alkaloids

Alkaloids exhibit basic character due to the presence of amine groups in their structure. Under acidic conditions, they become protonated and migrate as cations (with the exception of methylxanthines). At pH 2.5, the silica surface is largely protonated, minimizing the adsorption of positively charged analytes. Consequently, these compounds are able to migrate toward the detector despite the reduced electroosmotic flow (EOF) [24].

The separation of five alkaloid standards using the protocol I, is shown in Fig. 2A. Alkaloids exhibit variable maximum UV absorption depending on the substituents attached to the core structure. In this method, detection was performed at 220 nm, corresponding to the absorption of the furan ring [25]. Figure 2 (Protocol I) presents the alkaloids standard mixture (A) and the crude extract of *D. odorata* seeds (B), analyzed under the same conditions. Several metabolite peaks ionized at acidic pH were observed, which may correspond to alkaloids, amino acids, or small peptides. Histamine was used as the internal standard. Although alkaloids are commonly reported in plant matrices, no evidence of their presence in *D. odorata* seeds was found in the present study, which is consistent with previous phytochemical reports describing coumarins and phenolic compounds as the major constituents of this species.

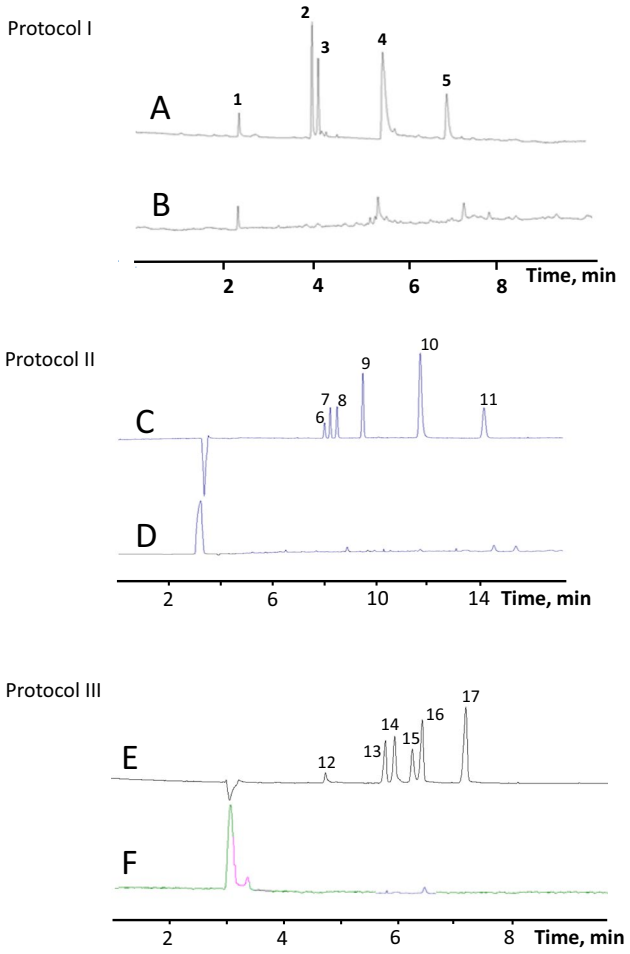

Fig. 2 CE direct-UV protocols. A, C and E – Reference standards at 20 mg L^{-1}, B, D and F—*D. odorata* seeds crude extract. Peak identification: PROTOCOL I: 1-histamine, 2-cinchonidine, 3-quinine, 4-scopolamine, 5-atropine. PROTOCOL II: 6-rutin, 7-naringenin, 8-scopoletin, 9-umbeliferone, 10-kaempferol, 11-quercetin. PROTOCOL III: 12-chlorogenic acid, 13-caffeic acid, 14-pherulic acid, 15- 6-hydroxybenzoic acid, 16- o-coumaric acid e 17-vanillic acid

Protocol II—investigation of flavonoids

When buffers at pH 8.5 or higher are used, the capillary surface becomes negatively charged, resulting in an increased EOF. Under these basic conditions, separation is governed by both the ionization of phenolic hydroxyl groups (pKa typically around 7–10) and the ability of borate to form complexes with cis-diol-containing compounds, particularly carbohydrates and polyphenols [26]. Although the analytes are predominantly present as anions, in the presence of EOF they are transported toward the detector. Phenolic compounds are typically analyzed using direct UV detection [15, 24]. Flavonoids exhibit characteristic UV absorption between 240 and 400 nm: bands at 240–280 nm are associated with the benzoyl system, whereas bands at 300–380 nm correspond to the cinnamoyl system [27]. Therefore, the UV/DAD detector should be set within this range to ensure analytical specificity. Under these conditions, phenolic compounds such as flavonoids and certain ionized coumarins can be effectively separated [14].

The separation of flavonoid standard mixture is shown in Fig. 2A (Protocol II). Four flavonoids and two coumarins were successfully separated in less than 15 min, showing high selectivity and resolution. The electropherogram of the *D. odorata* crude extract, analyzed under the same conditions, is present in Fig. 2B. Additional peaks were observed, corresponding to other compounds that were ionized at this pH. Coumarin, which remained un-ionized and migrated with the EOF, was used as the internal standard. None of the flavonoid or coumarin standards included in this protocol were detected in the crude extract.

Protocol III – investigation of phenolic acid

By adjusting the pH to 7.0, phenolic acids are predominantly present in their anionic form due to the deprotonation of the carboxylic group (pKa ≈ 4–5), which promotes their electrophoretic migration and separation within the capillary. A phosphate buffer at neutral pH was used in this protocol to

facilitate the separation of phenylpropanoids and phenolic acids. The detector was set at 214 nm (Fig. 2, Protocol III).

The separation of a phenolic acid standard mixture in this protocol is shown in Fig. 2A, and the electrophoretic profile of the *D. odorata* seed crude extract is shown in Fig. 2B. Among the investigated compounds, only o-coumaric acid was identified in the crude extract. Since *o*-coumaric acid is an ionic compound, it can be analyzed using both MEKC and CZE modes. In a previous study, coumarin and *o*-coumaric acid were quantified in *D. odorata* seeds using MEKC (ref). In the present study, the determination of o-coumaric acid was evaluated using a FSCE protocol developed for phenolic acids. Coumarin was used as the internal standard because, under the selected conditions, it co-migrated with the electroosmotic flow (EOF) and was not electrophoretically separated. This behavior is attributed to its chemical structure, which renders it neutral under FSCE conditions and therefore non-ionized. Furthermore, the identification of o-coumaric acid can be conducted in both MECK and FSCE modes, demonstrating the versatility of CE for quality control purposes.

The detection of o-coumaric acid in the analyzed extracts is consistent with previous reports [6, 8, 28] and may reflect the active metabolism of phenylpropanoids associated with coumarin biosynthesis in this species Griffiths (1962). Since o-coumaric acid is considered an important intermediate in coumarin biosynthesis pathways, its presence in the samples is expected.

CE protocols using indirect UV absorbance detection

Indirect UV detection is a well-established CE approach for the determination of analytes lacking native chromophores, particularly organic and fatty acids, although its performance is strongly dependent on background electrolyte optimization and matrix composition [29, 30].

Protocol IV- investigation of carboxylic acid

Protocol IV was developed for the separation of compounds with pKa values below 5. Within the pH range of 3–4, low-molecular-weight carboxylic acids are sufficiently ionized to allow effective separation [31]. Due to their high mobility in the anionic form, reversal of the EOF is required to achieve adequate resolution [21]. For this purpose, CTAB, a commonly used cationic surfactant was added to the buffer to reverse the EOF. Due to their minimal UV absorption, carboxylic acids require the incorporation of a chromophore into the background electrolyte for indirect detection. In this protocol, 3.5-DNB at pH 3.6 served both as the running electrolyte and as the chromophore. Under these conditions, the electrophoretic mobility of 3.5-DNB closely matches that of the analytes, minimizing electromigration dispersion, improving peak symmetry, and reducing migration times [31]. The separation of dicarboxylic (malic, malonic, aspartic and glutamic acids) and monocarboxylic (lactic) acids standards was completed in under 7 min (Fig. 3A, Protocol IV).

The crude extract analyzed under Protocol IV conditions is shown in Fig. 3B. Peak identification was initially performed by spiking. The presence of dicarboxylic acids in polar and mid-polar extracts of *D. odorata* seeds has been reported in the literature; however, these reports are generic and do not provide identification of individual compounds [8]. As this represents the first report of dicarboxylic acids in *D. odorata* seeds, compound identities were further confirmed by ESI–MS in negative mode. Dicarboxylic acids, including glutamic acid (m/z 146) and aspartic acid (m/z 132), and malic acid (m/z 133), were identified in the crude extract. The detection of low-molecular-weight carboxylic acids, provides additional insight into the metabolic profile of the seeds. These compounds are associated with central

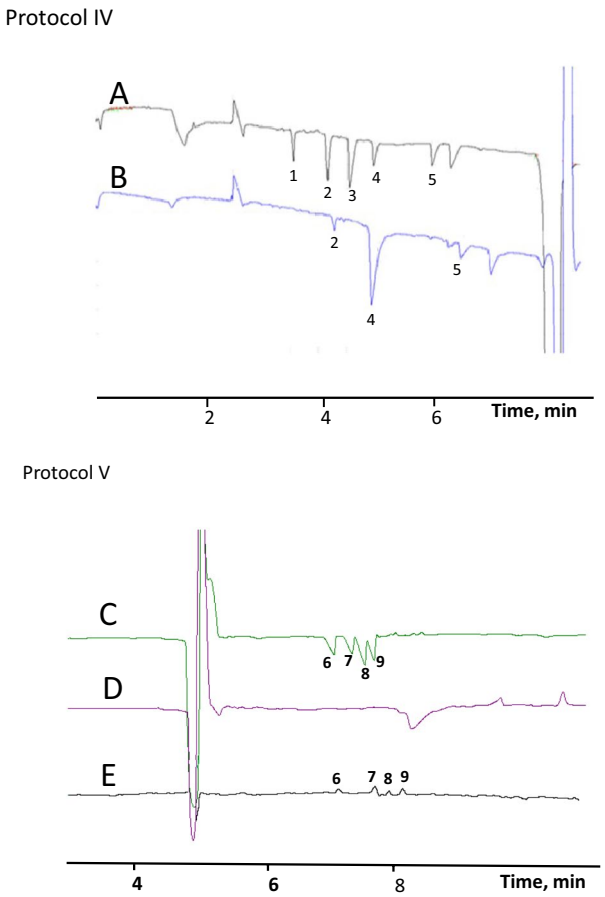

Fig. 3 CE indirect-UV protocols. A- Carboxylic acids standards at 25 mg L^{-1}. B and D—*D. odorata* seeds crude extract. C- Fatty acids standards at 250 mgL^{-1}; and E – saponified oil. Peak identification: PROTOCOL IV: 1-malonic acid, 2-aspartic, 3-latic acid, 4-malic acid, 5-glutamic acid. PROTOCOL V: 6-stearic acid, 7-oleic acid, 8-palmitic acid, 9-linoleic acid

biochemical pathways and may contribute to distinguishing *D. odorata* from other plant matrices, supporting its phytochemical characterization.

Protocol V—investigation of fatty acids

D. odorata seeds exhibited a high lipid content (≈27.4% dry weight), with oleic acid as the predominant fatty acid, followed by linoleic, palmitic, and stearic acids in lower proportions [32, 33]. This compositional profile is typical of oilseeds rich in long-chain fatty acids and directly influenced the electrophoretic behavior observed in the developed method. Since fatty acids (FAs) are weak acids with pKa values around 4.8–5.0, buffer pH is a critical parameter for separation. A pH above 7.0 ensured near-complete dissociation of the carboxyl groups, promoting migration in the anionic form. Under these conditions, FAs electrophoretically migrate toward the anode, but the electroosmotic flow (EOF) is sufficiently strong to transport the negatively charged species toward the cathode, enabling detection. The selected pH provided an optimal balance between migration time and resolution [33].

Due to the absence of intrinsic chromophores and their low UV absorptivity, indirect UV detection was used. Sodium dodecylbenzene sulfonate (SDBS) was selected as the background electrolyte chromophore because it provided improved baseline stability and sensitivity compared with previously reported systems using imidazole or quinine [19]. This choice contributed to enhanced detectability of all evaluated FAs. The addition of organic modifiers was essential to ensure FA solubility and prevent micelle formation. Acetonitrile, at approximately 45% (v/v), provided a suitable hydro-organic environment, reducing hydrophobic aggregation and contributing to reproducible migration times. Furthermore, the incorporation of selectivity modifiers improved resolution among structurally similar compounds [19]. 1-Octanol enhanced the separation of FAs with comparable chain lengths, such as palmitic and linoleic acids [34], while Brij 35 improved selectivity, particularly for cis–trans isomer discrimination.

Overall, the optimized conditions allowed efficient separation of the main fatty acids (FAs) present in *D. odorata* seeds, demonstrating the suitability of CE for this matrix. The separation of the available FA standards obtained under Protocol V conditions is shown in Fig. 3C, while the electropherogram of the crude *D. odorata* extract is presented in Fig. 3D.

Fatty acids in the seed extract may occur in either free or esterified forms. Therefore, CE analysis requires a prior saponification step to release these compounds in their free form [19]. Following saponification, stearic, oleic, palmitic, and linoleic acids were successfully identified (Fig. 3E). The fatty acid profile identified in *D. odorata* seeds, particularly the presence of oleic and linoleic acids as major constituents, is consistent with typical plant lipid compositions and may serve as a useful indicator for assessing the quality and authenticity of tonka bean-derived products.

Quantitative analysis of compounds in commercial Dipteryx odorata seed samples

Polar crude extracts obtained from commercial *D. odorata* seeds were directly injected under all evaluated protocols, demonstrating the selectivity of the CE-based methods for complex matrices. The results as representative of the analyzed commercial samples rather than the species as a whole. Variations in environmental conditions, geographical origin, genetic background, and post-harvest handling may influence the phytochemical profiling of seeds. It is worth noting that the use of commercially available, wildcrafted material reflects the actual products accessible to consumers and industry, which supports the practical relevance of the study.

The secondary metabolites identified in the polar extract were quantified, and the results are presented in Table 3. As the primary aim of this study was to evaluate CE-based protocols for establishing the chemical profile of compounds present in polar extracts of *D. odorata*, validation efforts were focused on the protocols in which the target analytes were successfully detected and separated. Accordingly, figures of merit were determined for Protocols III, IV, and

Table 3 Figures of merit and identified/quantified metabolites found in *D. odorata* seed crude extract

CE Protocols	Identified compounds	Range of linearity (mg L^{-1})	Determination coefficient (R^2)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Concentration (mg L^{-1})
Protocol I	n.i	-	-	-	-	-
Protocol II	n.i	-	-	-	-	-
Protocol III	o-coumaric acid*	0.2–4.0	0.991	0.55	1.03	11.19
Protocol IV	aspartic**	5–30	0.995	2.19	4.50	162.01
	malic**	3–20	0.991	3.41	6,10	10,99
	glutamic**	1–20	0.990	4.29	6.50	11.30
Protocol V	(C18:0) stearic*	3–20	0.990	2.50	5.13	6.27[#]
	(C18:1) oleic*	130–240	0.999	104.05	146.83	216.95[#]
	(C16:0) palmitic*	10–35	0.991	1.34	4.45	27.33[#]
	(C18:2) linoleic*	20–100	0.999	48.33	60.60	84.31[#]

n.i.—not identified; *expected compounds; **new compounds found. [#]—concentration found in the oil

V. The repeatability of the migration time showed an average coefficient of variation (CV) of 1.6%, indicating good method precision. Further studies may expand on these results by addressing full validation of all protocols.

Among the compounds previously reported in *D. odorata* seeds, FAs and o-coumaric acid were identified and quantified. In addition to quantification in mgL^{-1} (Table 3), the results were normalized and expressed as percentage composition to allow comparison with literature data. The o-Coumaric acid was quantified at 0.02% in the extract, which is consistent with values reported by Ehlers (0.07%) using HPLC–DAD analysis [6, 28]. Among the fatty acids, oleic acid was present at the highest concentration (21.69%), followed by linoleic (8.43%), stearic (6.27%), and palmitic acid (2.73%). This profile agrees with the fatty acid distribution commonly reported for *Dipteryx* seeds [8, 28, 35]. However, all quantified fatty acids were markedly lower-approximately twofold-than those reported by Cuchet et al. using HPLC–DAD for absolute extracts of *D. odorata*, with values of 40.93% for oleic, 14.95% for linoleic, 6.68% for palmitic, and 4.92% for stearic acid [28]. Stearic acid was the only exception, showing a slightly higher value in the present study (6.27%). These discrepancies likely reflect methodological differences, particularly the nanoliter injection volume in CE, the reduced capillary internal diameter relative to HPLC columns, and possible analyte losses during saponification.

As dicarboxylic acids are reported here for the first time in *D. odorata* seeds, and their quantification was further confirmed by MS. The concentrations obtained by MS (aspartic acid, 9.03 mg L^{-1}; malic acid, 20.58 mg L^{-1}; and glutamic acid, 10.45 mg L^{-1}) were in good agreement with those determined by CE-DAD (Table 3), corresponding to 0.16%, 0.41%, and 0.22% (w/w) for aspartic, malic, and glutamic acids, respectively. The predominance of malic acid suggests active carbon metabolism, while the relative proportions of glutamic and aspartic acids are consistent with their roles in nitrogen metabolism and amino acid biosynthesis. The quantification of low-molecular-weight carboxylic acids and amino acids, expands the current understanding of the chemical composition of *D. odorata* seeds beyond secondary metabolites. These compounds play key roles in primary metabolism and may serve as metabolic markers, contributing to a more comprehensive characterization of seed composition and potential implications for quality, authenticity, and sensory properties.

Conclusion

This study reports, for the first time, the application of CE-based protocols for the chemical profiling of the commercial polar extract of *D. odorata* seeds. The proposed methods demonstrated high selectivity, allowing the identification of specific phytochemical classes in a complex matrix without the need for sample pretreatment or fractionation. Fatty acids and *o*-coumaric acid were identified and quantified, with results consistent with concentrations previously reported using chromatographic techniques. Additionally, low-molecular-weight carboxylic acids and amino acids (malic, aspartic, and glutamic acids) were detected for the first time in this matrix, despite their low concentrations. In this context, the application of CE-based protocols represents a promising analytical approach for the simultaneous determination of structurally diverse compounds in complex matrices.

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Data availability The data presented in this study is available in article or supplementary material here.

Declarations

Competing interests The author(s) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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