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Chemical Profile and In Vitro Evaluation of the Antibacterial Activity of *Dioscorea communis* Berry Juice

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Abstract: Within the large family of Dioscoreaceae, *Dioscorea communis* (L.) Caddick & Wilkin (syn. *Tamus communis* L.) is considered among the four most widespread representatives in Europe, and it is commonly known under the name black bryony or bryonia. To date, reports have revealed several chemical components from the leaves and tubers of this plant. Nevertheless, an extensive phytochemical investigation has not been performed on its berry juice. In the present study, metabolite profiling procedures, using LC-MS, GC-MS, and NMR approaches, were applied to investigate the chemical profile of the *D. communis* berries. This work reveals the presence of several metabolites belonging to different phytochemical groups, such as fatty acid esters, alkylamides, phenolic derivatives, and organic acids, with lactic acid being predominant. In parallel, based on orally transmitted traditional uses, the initial extract and selected fractions were tested in vitro for their antibacterial effects and exhibited good activity against two bacterial strains related to skin infections: methicillin-resistant *Staphylococcus aureus* and *Cutibacterium acnes*. The MIC and MBC values of the extract were determined at 1.56% *w*/*v* against both bacteria. The results of this study provide important information on the chemical characterization of the *D. communis* berry juice, unveiling the presence of 71 metabolites, which might contribute to and further explain its specific antibacterial activity and its occasional toxicity.

Keywords: *Dioscorea communis*; Dioscoreaceae; GC-MC; LC-MS; NMR; antibacterial; *Staphylococcus aureus*; *Cutibacterium acnes*

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1. Introduction

The genus *Dioscorea* L. is the largest representative of the Dioscoreaceae family, consisting of ≤600 species [1]. The name of the genus, as well as the whole family, was given by the French botanist Charles Plumier in honor of the famous Greek physician Pedanios Dioscorides [2]. *Dioscorea* species are mainly distributed across wet and periodically dry tropical regions, whereas some of them are extended from temperate to alpine climates [3]. In European countries, the Dioscoreaceae family is represented only by four species: *Dioscorea balcanica* Košanin and the formerly known *Borderea pyrenaica* Miégeville

(syn. *Dioscorea pyrenaica* Bubani & Bordère ex Gren), *Borderea chouardii* (Gaussen) Heslot (syn. *Dioscorea chouardii* Gaussen), and *Tamus communis* L. (syn. *D. communis* L.) [4]. The previously recognized genera *Borderea* Miégeville and *Tamus* L. have been integrated into the genus *Dioscorea* due to the high morphological molecular similarities [1,5]. Among them, *Dioscorea* and *Borderea* species are distributed in the Balkan peninsula and the Pyrenees, while *D. communis* is endemic in South, southern Central, and West Europe toward Northern England [4].

D. communis is a perennial herbaceous climber with large tubers and red berries distributed in the Mediterranean area. Its young stems are part of the traditional diet of many Mediterranean countries, such as Greece, Spain, Portugal, Italy, and Croatia, as a kind of wild asparagus [6]. The plant material is edible only before the flowering period and after cooking, contrary to the completely grown plant considered not edible due to its toxic effects. The species has a great number of synonyms, which is attributed to the high diversity of its phenotype. The most common names are black bryony, bryonia, abronya, or herbe aux femmes battues [6–8]. Different parts of the plant have been used in folk medicine, including the fruits and the roots, which have been applied externally for the treatment of musculoskeletal abnormalities and rheumatism, as well as skin diseases, injuries, and bruises due to their analgesic and rubefacient effect [6,7,9,10]. It is worth mentioning that *D. communis* has been reported (by oral sources) to be used as a traditional remedy in southern Central Greece for skin-related ailments, as well as against oral infections. Previous phytochemical studies on *D. communis* have revealed the presence of saponins [11], phenanthrenes [12], sterols [11], and flavonoid glycosides [13] in the aerial parts and tubers. Regarding bioactivity, *D. communis* has been found to exhibit primarily antioxidant [14,15] and anti-inflammatory effects in vitro and in vivo [16]. Additionally, all parts of the plant are associated with toxicity due to the presence of saponins, calcium oxalate crystals, and histamine. When the berry juice or roots are in contact with skin, they can cause erythematous and papular rash, since calcium oxalate and histamine show high cutaneous penetration and induce allergic reactions [7,17].

Dioscorea spp., aside from anti-inflammatory, antioxidant [18], antitumor [19], and neuroprotective activity [20], have also shown great antimicrobial activity. Kuete et al. (2012) investigated the antibacterial activity of the methanol extract of the air-dried bulbils of *D. bulbifera* L. var sativa against mycobacteria and multidrug-resistant, Gram-negative bacteria. The results indicated the strong antimicrobial activity of the crude extract against *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, and *M. tuberculosis*, with the MIC rising to 64 μg/mL in all tested bacterial strains [21].

Taking these into consideration, the aim of the present study is extensive phytochemical research on the berry juice of *D. communis* using various approaches, including chromatographic methods (CC, LC-MS, and GC-MS) and direct spectroscopic methods (NMR). A further goal is assessment of the antibacterial properties against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Cutibacterium acnes*, both associated with skin diseases.

2. Materials and Methods

2.1. Plant Material

The aerial parts from *D. communis* were collected from cultivated populations in Stamata of North Attica (Central Greece) (coordinates (WGS84): latitude: 38°08′22.3″ N; longitude: 23°53′09.9″ E) during the flowering stage in June 2019. The collected plant material was recognized and authenticated by Prof. Th. Constantinidis (Faculty of Biology, NKUA). A voucher specimen was deposited in the Department of Pharmacognosy and Chemistry of Natural Products (Faculty of Pharmacy, NKUA) under the code Skaltsa and Rallis 001.

2.2. General Experimental Procedures

The NMR spectra were measured in an AVANCE III 600 (Bruker, Corporation, Billerica, MA, USA) instrument equipped with a 5-mm TBI CryoProbe (^{1}H-NMR 600 MHz, ^{13}C-NMR 150 MHz) or a Bruker DRX 400 (^{1}H-NMR 400 MHz, Bruker manufacturer, Corporation, Billerica, MA, USA) at 298 K. Chemical shifts were given in ppm (δ) and were referenced to the solvent signals at 3.31/49.0 ppm for MeOD and 7.24/77.0 ppm for $CDCl_3$. Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Correlation (HSQC), and Heteronuclear Multiple Bond Correlation (HMBC) experiments were performed using standard Bruker microprograms. The HRESIMS spectra were obtained with an Agilent MS Q-TOF G6540A spectrometer (Santa Clara, CA, USA). Column chromatography (CC) was performed on a silica gel (Merck, Darmstadt, Germany, Art. 7736; Merck, Art. 9385) or Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO, USA). Fractionation was always monitored by TLC silica gel 60 F-254 (Merck, Darmstadt, Germany, Art. 5554) and cellulose (Merck, Darmstadt, German, Art. 5552) with visualization under UV (254 and 365 nm) and spraying with vanillin-sulfuric acid reagent (Merck, Darmstadt, Germany, Art. S26047 841), as well as Neu's reagent for phenolics (Alfa Aesar, Karlsruhe, Germany, A16606) [22]. Medium-pressure liquid chromatography (MPLC) support consisted of a reversed-phase column (Merck, Darmstadt, Germany, 10167) of 36 × 3.6 cm (Büchi Borosilikat 3.3, Flawil, Switzerland19674) on a system Büchi Pump C-615 and with a flow rate of 1 mL/min. The lyophilizer was Christ, ALPHA I-5 (Apeldoorn, Netherlands). All obtained extracts, fractions, and isolated compounds were evaporated to dryness in a vacuum under low temperature and then were put in activated desiccators with P_2O_5 until their weights had stabilized.

2.3. Extraction, Fractionation, and Isolation

The plant material (berry juice, 1 L) was initially processed by lyophilization to yield a dry residue (50.0 g). Different extraction procedures were applied for further separation or isolation. A part of the lyophilized berry juice (A; 4.0 g) was pre-fractioned by RP_{18}-MPLC using mobile phase mixtures of H_2O:methanol (MeOH) (from 100:0 to 0:100) of decreasing polarity and gradient elution (flow rate: 1 mL/min) to finally yield 9 fractions of 900 mL each (AA-AI). The fractions were evaporated at reduced pressure below 50 °C, and afterward, they were monitored by ^{1}H-NMR. Fraction AD (396.4 mg, eluted with H_2O:MeOH (80:20)) was subjected to column chromatography (CC) over silica gel using mixtures of cyclohexane (CyHex):dichloromethane and (CH_2Cl_2):MeOH:H_2O of increasing polarity as eluents to give 66 fractions, which were combined based on TLC similarities into 33 groups ($AD_{(A–G)}$). The subfraction AD_B (8.9 mg, eluted with CyHex:CH_2Cl_2 (30:70)) yielded compound **3** in a mixture with compound **4** and methyl stearate. Fraction AF (60.0 mg, eluted with H_2O:MeOH (60:40)) was subjected similarly to CC over silica gel (CyHex:CH_2Cl_2:MeOH (from 100:0:0:0 to 0:0:100)) to give 33 fractions, which were combined based on TLC similarities into 13 groups ($AF_{(A–M)}$). The subfraction AF_A (10.3 mg, eluted with CyHex:CH_2Cl_2 (30:70)) yielded compound **3** in a mixture with **4**. Another part of the lyophilized berry juice (A′, 1.65 g) was subjected to further fractionation by CC over silica gel. Mixtures of CyHex, CH_2Cl_2, MeOH, and H_2O with increasing polarity were used as eluents to give 44 fractions, which were combined based on TLC similarities into 20 groups ($A'_{(A–T)}$). Among them, subfraction A′G was identified as compound **4** (5.2 mg). Furthermore, combined subfractions A′L, A′M, and A′N (A′L′; 90.0 mg, eluted with CH_2Cl_2:MeOH:H_2O (60:40:4.0–55:45:4.5)) were subjected to CC over Sephadex LH-20 (MeOH 100%) and yielded 34 fractions, which were combined based on TLC similarities into 12 groups ($A'L'_{(A–L)}$). Subfraction $A'L'_I$ was identified as compound **5** (18.4 mg) [23].

Moreover, two equal amounts (4.0 g) of the lyophilized berry juice (A1 and A2) were subjected to liquid-liquid extraction to extract the non-polar constituents in two different ways. A1 was dissolved in H_2O (10 mL), and the aqueous layer was extracted with diethyl ether (Et_2O; 10 mL × 3; A1A) and then with CH_2Cl_2 (10 mL × 3; A1B). A2 was first subjected to acid hydrolysis by boiling under reflux with 10% w/v hydrochloric acid (37%

w/*w*) for 120 min, and then it was extracted as described above with Et_2O (A2A) and CH_2Cl_2 (A2B). The organic layers of all extractions were concentrated to dryness, and afterward, they were analyzed by GC-MS and ^{1}H-NMR. The ^{1}H-NMR analyses revealed the presence of compound **1** in all obtained extracts, while compound **2** was found only in A2A and A2B. In addition, 80.0 mg of the lyophilized berry juice (A3) was extracted with *n*-butanol, and the organic phase, after evaporation, was analyzed by LC-MS. The flow chart of the isolation procedures is shown in Figure S40 [23].

2.4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Fractions A1A, A1B, A2A, and A2B, as well as the less polar AE, AG, and AI, were subjected to GC-MS analyses using a Hewlett-Packard 7820A-5977B MSD system (Agilent Technologies, Santa Clara, CA, USA) operating in EI mode (70 eV), equipped with an HP-5MS-fused silica capillary column (30 m × 0.25 mm; film thickness: 0.25 µm) and a split-splitless injector. The temperature program was, from 60 °C at the time of injection, raised to 300 °C at a rate of 3 °C/min and subsequently held at 300 °C for 10 min. Helium was used as a carrier gas at a flow rate of 2.0 mL/min. The injected volume of the samples was 2 µL [24].

The retention indices for all compounds were determined according to the Van der Dool approach [25], with reference to a homologous series of *n*-alkanes from C_9 to C_{25}. The identification of the chemical components was based on a comparison of both relative retention times and mass spectra with those reported by Adams [26] and the NIST/NBS and Wiley libraries. The component relative percentages were calculated based on the GC peak areas without using correction factors [24].

2.5. Liquid Chromatography High-Resolution Quadrupole Time-of-Flight Mass Spectrometry (LC-Q-TOF-MS/MS)

Analyses of the butanol extract of berry juice (A3) and of selected fractions were performed with a UHPLC Agilent 1290 infinity system with a DAD G4212A and MS Agilent G6540A Q-TOF with Agilent Jet Stream technology electrospray ionization. Separation was performed on a Phenomenex Luna Omega column (C18, 1.9 u, 90 A°, 75 × 2.0 mm) using gradient mixtures of 0.1% formic acid (solvent A) and MeCN supplemented with 0.1% formic acid (solvent B) (gradient: 0.0−8.0 min, 0%→30% B; 8.0−8.1 min, 30%→98% B; 8.1−9.1 min, 98% B; 9.1−9.2 min, 98%→5% B; 9.2−10.0 min, 5% B; flow rate: 0.6 mL/min; injection volume: 1 µL; oven temperature: 40 °C). Data analysis was performed by MassHunter Workstation Software Qualitative Analysis (B.07.00, Agilent) using automatic mass spectrum integration. LC-Q-TOF-MS/MS analyses were performed in positive and negative ionization modes to obtain the maximum information on its composition. The metabolites were characterized based on their mass spectra using the precursor ion and comparison of the fragmentation patterns with molecules described in the literature [27].

2.6. Nuclear Magnetic Resonance Spectroscopy (NMR) Spectroscopy

During the whole analysis course, all extracts and obtained subfractions were continuously monitored and traced down using an NMR metabolomic strategy, which permitted detailed characterization thereof. Furthermore, the NMR spectra of compounds **1**–**5** were measured (Figures S19–S33), as well as of the fractions with low complexity (AE, AG, and AI).

2.7. Identification of Cutibacterium acnes Strain ATCC 6919 by 16S rRNA Gene Sequencing

Genomic DNA extraction from Pure BHI broth and Anaerobe CDC Blood agar cultures of *Cutibacterium acnes* strain ATCC 6919 was performed using an ExtractMe Genomic DNA Kit (Blirt, Gdánsk, Poland). Universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) [28] and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [29] (Eurofins Genomics, Germany) were used to amplify the 16S rRNA gene by PCR. The reaction mixture contained the following: 1 U FastGene Taq DNA Polymerase (NIPPON Genetics, Tokyo, Japan), 1 × PCR buffer A,

25 pmol of each primer, 1 mM dNTPs, a 3µL DNA template, and deionized sterile water at a final volume of 50 µL. The thermal cycler Primus 25 (PEQLAB Biotechnologie, Erlangen, Germany) was used in the following PCR conditions: initialization at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 2 min. A final elongation step at 72 °C for 5 min was added.

Amplicons of *C. acnes* were purified using the NucleoSpin Gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany) and then directly sequenced via the Sanger dideoxy termination method by Cemia (Larissa, Greece). Chromas (Version 2.6.6 Software, Technelysium Pty Ltd., South Brisbane, Australia, www.technelysium.com.au, accessed on 20 October 2021) was used to check the quality of the obtained sequencing results. The sequences were assembled into a single sequence via MEGA X (Version 10.1.6 Software) [30] and Gene Runner (Version 6.5 Software, Inc., Hudson, NY, USA, www.generunner.net accessed on 20 October 2021) and subjected to a BlastN (Megablast) (Bethesda, MD, USA, https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 20 October 2021) search in the 16S rRNA Database-GENEBANK to identify the sequences with the highest similarity.

2.8. Bacterial Strains and Growth Conditions

The antibacterial activity of *D. communis* berry juice was determined against MRSA strain 1552 and *Cutibacterium acnes* strain ATCC 6919. MRSA strain 1552 was isolated from the clinical samples, and the identification and characterization were conducted by standard laboratory methods (kindly provided by Prof. Spyros Pournaras, School of Medicine, NKUA). MRSA was routinely grown in Müller–Hinton broth (Lab M, Bury, UK) or Müller–Hinton agar (Lab M, Bury, UK) at 37 °C aerobically and *C. acnes* in Brain Heart Infusion (BHI) broth (Condalab S.A., Spain) or BHI agar (Condalab S.A., Spain) at 37 °C anaerobically.

2.9. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the berry juice and AC-AG fractions were determined in sterile 96-well polystyrene microtiter plates (Kisker Biotech GmbH & Co. KG, Steinfurt, Germany) using a spectrophotometric bioassay as previously described [31], with some modifications. Briefly, 0.25 g of berry juice was suspended in sterile ddH_2O (2-mL final volume) for 1 h at room temperature with occasional vortexing and then centrifuged at 10,000× *g* for 7 min. The aqueous phase was filtered through a 0.22-µm syringe filter and used for serial dilutions (in Müller–Hinton and BHI broth for MRSA and *C. acnes*, respectively), corresponding from 25 to 0.39% *w*/*v*. The weighed part of the AC-AG fractions was suspended in sterile ddH_2O containing 1.5% DMSO (2.5-mL final volume) and then centrifuged at 5000× *g* for 3 min. The aqueous phase was filtered through a 0.22-µm syringe filter and used for serial dilutions as described above. Overnight bacterial cultures of MRSA (grown in Müller–Hinton) were adjusted to a 0.5 McFarland turbidity standard (~1.5 × 10^8 CFU/mL). For 3 days, the old bacterial cultures of *C. acnes* (grown in BHI broth) were adjusted to a 0.5 McFarland turbidity standard (~1.5 × 10^8 CFU/mL). A 10-µL broth, containing approximately 5 × 10^4 CFUs, was added to 190 µL of the tested twofold sample dilutions.

The positive control wells, containing broth, were inoculated with MRSA or *C. acnes* to test the growth of the pathogen. The negative control wells contained dilutions of berry juice or fractions in Müller–Hinton or BHI broth without bacteria. The Müller–Hinton or BHI broth control wells without bacteria were used to test for any possible contamination.

The optical density (OD) was determined at 600 nm using an EL × 808 absorbance microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) just prior to incubation (t = 0) and 24 h after incubation (t = 24 h) at 37 °C aerobically for MRSA (t = 0) and 5 days after incubation (t = 5 d) at 37 °C for *C. acnes* under anaerobic conditions. The OD for each negative control replicate well (containing sample) at t = 24 or t = 5 d for MRSA and *C. acnes* was subtracted from the OD of the same replicate test well with bacteria at t = 24 or t = 5 d for MRSA and *C. acnes*, respectively. The growth inhibition at each sample dilution was determined using the formula

% inhibition = [1 − (OD test well − OD of corresponding negative control well)] × 100. The MIC was determined as the lowest sample concentration which resulted in 100% growth inhibition. The MIC values of the berry juice and AC-AG fractions were expressed as w/v and mg/mL, respectively.

2.10. Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by transferring a small quantity of the sample contained in each replicate well of the microtiter plates to Müller–Hinton agar plates for MRSA and BHI agar for *C. acnes* by using a microplate replicator (Boekel Scientific, Feasterville-Trevose, PA, USA). The plates were incubated for 24 h aerobically for MRSA and 5 days anaerobically for *C. acnes* at 37 °C. The MBC was determined as the lowest concentration of the initial extract and fractions at which no grown colonies were observed [32].

3. Results and Discussion

Previous studies on the title plant showed that root tubers have been the most intensively investigated plant part, with triterpenoids, sterols, and saponins as well as phenanthrenes and furanocoumarins being reported [11,12,33,34]. Similarly, the aerial parts (leaves and shoots) have resulted in a different yield of phenolic derivatives and flavonoids, saponins, sterols, triterpenoids, carotenoids, tocopherols, fatty acids, and organic acids [11,15,35,36]. Nevertheless, to the best of our knowledge, extracts from the berry juice led to the identification of sterols and flavonoids in the *O*- and *C*-glucoside forms, though no extensive phytochemical investigation has been performed [16,37]. In southern Central Greece, *D. communis* berry juice has been reported (by oral sources) as a traditional remedy for skin-related ailments, as well as for oral infections. Taking into consideration the above, our study was oriented to the investigation of the berry juice, targeting its phytochemical content and its potential biological effects on bacteria related to skin infections.

The phytochemical analysis of *D. communis* berry juice was processed by different chromatographic techniques. Through GC-MS and LC-MS/MS chromatographies aided by NMR spectroscopy, a great number of compounds belonging to different phytochemical groups was identified. The results are presented in Tables 1–8.

Table 1. Chemical composition of diethyl ether extract of *Dioscorea communis* berry juice (A1A).

No.	Retention Time	% Area	KI	AI	Name of Compound	Molecular Formula	MW
1	39.611	6.3	1950	1964	1,2-benzenedicarboxylic acid, dibutyl ester [dibutyl phthalate]	$C_{16}H_{22}O_4$	278
2	41.018	3.0	1993	1992	hexadecanoic acid, ethyl ester [ethyl palmitate]	$C_{18}H_{36}O_2$	284
3	44.032	2.1	2089	2095	9Z,12Z-octadecadienoic acid, methyl ester [methyl linoleate]	$C_{19}H_{34}O_2$	294
4	46.097	45.2	2157	2159	9Z,12Z-octadecadienoic acid, ethyl ester [ethyl linoleate]	$C_{20}H_{36}O_2$	308
5	46.272	36.9	2163	2173	9Z,12Z,5Z-octadecatrienoic acid, ethyl ester [ethyl linolenate]	$C_{20}H_{34}O_2$	306
6	46.515	3.7	2171	2179	9Z-octadecenoic acid, ethyl ester [ethyl oleate]	$C_{20}H_{38}O_2$	310
7	70.332	2.8		3130	α-tocopherol	$C_{29}H_{50}O_2$	430
	Total	100.0					

Table 2. Chemical composition of dichloromethane extract of *Dioscorea communis* berry juice (A1B).

No.	Retention Time	% Area	KI	AI	Name of Compound	Molecular Formula	MW
1	41.014	3.3	1993	1992	hexadecanoic acid, ethyl ester (ethyl palmitate)	$C_{18}H_{36}O_2$	284
2	44.025	2.7	2089	2095	9*Z*,12*Z*-octadecadienoic acid, methyl ester (methyl linoleate)	$C_{19}H_{34}O_2$	294
3	44.206	tr	2094	2105	9*Z*,12*Z*,15*Z*-octadecatrienoic acid, methyl ester (methyl linolenate)	$C_{19}H_{32}O_2$	292
4	46.095	46.7	2157	2159	9*Z*,12*Z*-octadecadienoic acid, ethyl ester (ethyl linoleate)	$C_{20}H_{36}O_2$	308
5	46.271	38.2	2163	2173	9*Z*,12*Z*,15*Z*-octadecatrienoic acid, ethyl ester (ethyl linolenate)	$C_{20}H_{34}O_2$	306
6	46.509	3.6	2170	2179	9*Z*-octadecenoic acid, ethyl ester (ethyl oleate)	$C_{20}H_{38}O_2$	310
7	70.328	2.9		3130	α-tocopherol	$C_{29}H_{50}O_2$	430
8	73.784	2.6		3203	(3β)-stigmast-5-en-3-ol (β-sitosterol)	$C_{29}H_{50}O$	414
	Total	100.0					

Table 3. Chemical composition of diethyl ether extract of *Dioscorea communis* berry juice after acid hydrolysis (A2A).

No.	Retention Time	% Area	KI	AI	Name of Compound	Molecular Formula	MW
1	37.969	tr	1900	1890	9*Z*-hexadecenoic acid, methyl ester (methyl palmitoleate)	$C_{17}H_{32}O_2$	268
2	38.823	53.3	1926	1921	hexadecanoic acid, methyl ester (methyl palmitate)	$C_{17}H_{34}O_2$	270
3	44.038	20.3	2089	2095	9*Z*,12*Z*-octadecadienoic acid, methyl ester (methyl linoleate)	$C_{19}H_{34}O_2$	294
4	44.202	14.8	2094	2105	9*Z*,12*Z*,15*Z*-octadecatrienoic acid, methyl ester (methyl linolenate)	$C_{19}H_{32}O_2$	292
5	44.435	1.3	2102	2103	9*Z*-octadecenoic acid, methyl ester (methyl oleate)	$C_{19}H_{36}O_2$	296
6	45.142	3.5	2125	2124	octadecanoic acid, methyl ester (methyl stearate)	$C_{19}H_{38}O_2$	298
7	46.087	3.2	2156	2159	9*Z*,12*Z*-octadecadienoic acid, ethyl ester (ethyl linoleate)	$C_{20}H_{36}O_2$	308
8	46.274	2.2	2163	2173	9*Z*,12*Z*,15*Z*-octadecatrienoic acid, ethyl ester (ethyl linolenate)	$C_{20}H_{34}O_2$	306
9	47.570	1.5	2206		unknown (m/z = 278.3)		
10	51.039	tr	2324	2329	eicosanoic acid, methyl ester (methyl arachidate)	$C_{21}H_{42}O_2$	326
	Total	98.5					

Table 4. Chemical composition of dichloromethane extract of *Dioscorea communis* berry juice after acid hydrolysis (A2B).

No.	Retention Time	% Area	KI	AI	Name of Compound	Molecular Formula	MW
1	38.823	33.2	1926	1921	hexadecanoic acid, methyl ester (methyl palmitate)	$C_{17}H_{34}O_2$	270
2	41.061	tr	1995	1992	hexadecanoic acid, ethyl ester (ethyl palmitate)	$C_{18}H_{36}O_2$	284
3	44.059	7.7	2090	2095	9*Z*,12*Z*-octadecadienoic acid, methyl ester (methyl linoleate)	$C_{19}H_{34}O_2$	294
4	44.280	6.8	2097	2105	9*Z*,12*Z*,15*Z*-octadecatrienoic acid, methyl ester (methyl linolenate)	$C_{19}H_{32}O_2$	292
5	45.217	tr	2128	2124	octadecanoic acid, methyl ester (methyl stearate)	$C_{19}H_{38}O_2$	298
6	46.117	28.1	2157	2159	9*Z*,12*Z*-octadecadienoic acid, ethyl ester (ethyl linoleate)	$C_{20}H_{36}O_2$	308
7	46.315	24.2	2164	2173	9*Z*,12*Z*,15*Z*-octadecatrienoic acid, ethyl ester (ethyl linolenate)	$C_{20}H_{34}O_2$	306
	Total	100.0					

Table 5. Chemical composition of fraction AI.

No.	Retention Time	% Area	KI	AI	Name of Compound	Molecular Formula	MW
1	7.787	2.3	1102	1100	nonanal (pelargonaldehyde)	$C_9H_{18}O$	142
2	27.237	5.3	1598	1600	hexadecane	$C_{16}H_{34}$	226
3	34.204	1.9	1791	1803	3-hexadecanone	$C_{16}H_{32}O$	240
4	34.930	4.9	1812	1811	hexadecanal (palmitaldehyde)	$C_{16}H_{32}O$	240
5	35.956	1.6	1842	1845	6,10,14-trimethyl-2-pentadecanone (hexahydrofarnesyl acetone)	$C_{18}H_{36}O$	268
6	38.238	9.4	1909	1902	2*E*-nonadecene	$C_{19}H_{38}$	266
7	38.398	5.0	1914		unknown (m/z = 266.1)		
8	38.750	2.4	1924	1921	hexadecanoic acid, methyl ester (methyl palmitate)	$C_{17}H_{34}O_2$	270
9	41.213	34.0	1999	2004	2-octadecanone	$C_{18}H_{36}O$	268
10	41.724	3.0	2016	2013	octadecanal (stearaldehyde)	$C_{18}H_{36}O$	268
11	43.737	1.3	2079	2077	1-octadecanol (stearyl alcohol)	$C_{18}H_{38}O$	270
12	44.068	1.7	2090	2095	9*Z*,12*Z*-octadecadienoic acid, methyl ester (methyl linoleate)	$C_{19}H_{34}O_2$	294
13	44.242	2.6	2095	2103	9*Z*-octadecenoic acid, methyl ester (methyl oleate)	$C_{19}H_{36}O_2$	296
14	45.053	3.0	2122		unknown (m/z = 282.1)		
15	45.133	2.2	2125	2124	octadecanoic acid, methyl ester (methyl stearate)	$C_{19}H_{38}O_2$	298
16	45.667	1.5	2143	2141	9*Z*-octadecenoic acid (oleic acid)	$C_{18}H_{34}O_2$	282
17	46.618	2.9	2174		unknown (m/z = 283.3)		
18	47.256	2.3	2195	2196	octadecanoic acid, ethyl ester (ethyl stearate)	$C_{20}H_{40}O_2$	312
19	47.395	6.3	2200	2200	docosane	$C_{22}H_{46}$	310
20	50.300	1.2	2298	2300	tricosane	$C_{23}H_{48}$	324
21	51.307	0.9	2332	2329	eicosanoic acid, methyl ester (methyl arachidate)	$C_{21}H_{42}O_2$	326
22	51.720	2.0	2347		unknown (m/z = 323.3)		
23	53.078	2.3	2393	2400	tetracosane	$C_{24}H_{50}$	338
	Total	89.1					

Table 6. Chemical composition of fraction AE.

No.	Retention Time	% Area	KI	AI	Name of Compound	Molecular Formula	MW
1	34.435	7.1	1797	1800	2-hexadecanone	$C_{16}H_{32}O$	240
2	35.344	1.7	1824	1826	pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256
3	37.890	3.2	1898	1901	2-heptadecanone	$C_{17}H_{34}O$	254
4	38.767	3.8	1925	1921	hexadecanoic acid, methyl ester (methyl palmitate)	$C_{17}H_{34}O_2$	270
5	41.437	49.4	2007	2004	2-octadecanone	$C_{18}H_{36}O$	268
6	42.043	1.7	2026	2028	heptadecanoic acid, methyl ester (methyl margarate)	$C_{18}H_{38}O_2$	284
7	44.112	4.2	2091	2095	9*Z*,12*Z*-octadecadienoic acid, methyl ester (methyl linoleate)	$C_{19}H_{34}O_2$	294
8	45.180	20.8	2126	2124	octadecanoic acid, methyl ester (methyl stearate)	$C_{19}H_{38}O_2$	298
9	45.713	4.8	2144	2141	9*Z*-octadecenoic acid (oleic acid)	$C_{18}H_{34}O_2$	282
10	47.281	3.3	2196		unknown (m/z = 313.2)		
	Total	96.7					

Table 7. Chemical composition of fraction AG.

No.	Retention Time	% Area	KI	AI	Name of Compound	Molecular Formula	MW
1	34.928	tr	1812	1811	hexadecanal (palmitaldehyde)	$C_{16}H_{32}O$	240
2	37.855	0.6	1897	1901	2-heptadecanone	$C_{17}H_{34}O$	254
3	38.228	2.5	1909	1902	2*E*-nonadecene	$C_{19}H_{38}$	266
4	38.358	1.6	1913		unknown (m/z = 266.1)		
5	38.734	1.3	1924	1921	hexadecanoic acid, methyl ester (methyl palmitate)	$C_{17}H_{34}O_2$	270
6	40.385	1.0	1975		unknown (m/z = 285.1)		

Table 7. *Cont.*

No.	Retention Time	% Area	KI	AI	Name of Compound	Molecular Formula	MW
7	41.007	tr	1994	1992	hexadecanoic acid, ethyl ester (ethyl palmitate)	$C_{18}H_{36}O_2$	284
8	41.372	59.7	2005	2004	2-octadecanone	$C_{18}H_{36}O$	268
9	41.690	2.4	2015	2013	octadecanal (stearaldehyde)	$C_{18}H_{36}O$	268
10	42.013	0.7	2025	2028	heptadecanoic acid, methyl ester (methyl margarate)	$C_{18}H_{38}O_2$	284
11	42.778	1.4	2050		unknown (m/z = 282.1)		
12	43.568	1.6	2075		unknown (m/z = 299.1)		
13	45.163	11.5	2126	2124	octadecanoic acid, methyl ester (methyl stearate)	$C_{19}H_{38}O_2$	298
14	46.080	0.4	2157	2159	9*Z*,12*Z*-octadecadienoic acid, ethyl ester (ethyl linoleate)	$C_{20}H_{36}O_2$	308
15	46.646	11.6	2175		unknown (m/z = 313.3)		
16	47.386	0.5	2200	2200	docosane	$C_{22}H_{46}$	310
17	71.869	0.5		3131	(3β,24*R*)-ergost-5-en-3-ol (campesterol)	$C_{28}H_{48}O$	410
18	72.575	0.8		3170	(3β,22*E*)-stigmasta-5,22-dien-3-ol (stigmasterol)	$C_{29}H_{48}O$	412
19	73.781	1.9		3203	(3β)-stigmast-5-en-3-ol (β-sitosterol)	$C_{29}H_{50}O$	414
	Total	82.8					

Components are listed in all tables according to their elution from an HP-5MS column. KI = Kováts indices calculated against C_9-C_{25} *n*-alkanes on the HP-5MS column; AI = arithmetic indices; and tr = traces.

Table 8. LC-MS of butanol extract.

Positive Ion Mode			Negative Ion Mode		Molecular Formula	Metabolite Name
RT	Found	Calcd	Found	Calcd		
0.324	203.0530 $[M + Na]^+$	203.0526	179.0563 $[M - H]^-$	179.0561	$C_6H_{12}O_6$	hexose
0.337	365.1059 $[M + Na]^+$	365.1054	341.1093 $[M - H]^-$	341.1089	$C_{12}H_{22}O_{11}$	hexose-pentose
0.488	113.0217 $[M + Na]^+$	113.0209	89.0243 $[M - H]^-$	89.0244	$C_3H_6O_3$	lactic acid
0.490	349.1121 $[M + Na]^+$	349.1105	371.1189 $[M + HCOO]^-$	371.1195	$C_{12}H_{22}O_{10}$	hexose-pentose
0.559			175.025 $[M - H]^-$	175.0248	$C_6H_8O_6$	ascorbic acid
0.565	117.0182 $[M + H]^+$	117.0182			$C_4H_4O_4$	fumaric acid
0.605	121.0652 $[M + H]^+$	121.0648			C_8H_8O	2-methylbenzaldehyde
0.917	166.0866 $[M + H]^+$	166.0863	164.0716 $[M - H]^-$	164.0717	$C_9H_{11}NO_2$	phenylalanine
1.208	146.0604 $[M + H]^+$	146.0600			C_9H_7NO	4-formyl indole
1.210	208.0609 $[M + H]^+$	208.0604	206.0458 $[M - H]^-$	206.0459	$C_{10}H_9NO_4$	pyranonigrin S
1.303	239.1489 $[M + H]^+$	239.1489			$C_{10}H_{22}O_6$	unknown
1.404	188.0707 $[M + H]^+$	188.0706			$C_{11}H_9NO_2$	unknown
1.725	247.1080 $[M + H]^+$	247.1077			$C_{13}H_{14}N_2O_3$	unknown
1.794	217.0977 $[M + H]^+$	217.0972			$C_{12}H_{12}N_2O_2$	unknown
1.973	231.1131 $[M + H]^+$	231.1128	229.0983 $[M - H]^-$	229.0983	$C_{13}H_{14}N_2O_2$	unknown
1.983	611.1609 $[M + H]^+$	611.1607	609.1462 $[M - H]^-$	609.1461	$C_{27}H_{30}O_{16}$	rutin
2.046	181.0498 $[M + H]^+$	181.0495	179.0350 $[M - H]^-$	179.0350	$C_9H_8O_4$	caffeic acid
2.175	275.1032 $[M + H]^+$	275.1026			$C_{14}H_{14}N_2O_4$	unknown
2.183	595.1668 $[M + H]^+$	595.1657	593.1516 $[M - H]^-$	593.1512	$C_{27}H_{30}O_{15}$	kaempferol 3-*O*-rutinoside
2.490			165.0556 $[M - H]^-$	165.0557	$C_9H_{10}O_3$	phenolic
3.006	349.1646 $[M + H]^+$	349.1646	347.1498 $[M - H]^-$	347.1500	$C_{19}H_{24}O_6$	unknown
3.248			345.1341 $[M - H]^-$	345.1644	$C_{19}H_{22}O_6$	unknown
4.183	883.4695 $[M + H]^+$	883.4686			$C_{45}H_{70}O_{17}$	7-oxodioscin
4.192	399.1781 $[M + Na]^+$	399.1778	375.181 $[M - H]^-$	375.1813	$C_{21}H_{28}O_6$	3,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptane
4.204	303.0502 $[M + H]^+$	303.0499	301.0353 $[M - H]^-$	301.0354	$C_{15}H_{10}O_7$	quercetin
4.714	279.2325 $[M + H]^+$	279.2319			$C_{18}H_{30}O_2$	linolenic acid
4.874	287.0551 $[M + H]^+$	287.0550	285.0406 $[M - H]^-$	285.0405	$C_{15}H_{10}O_6$	kaempferol
5.050			329.2333 $[M - H]^-$	329.2333	$C_{18}H_{34}O_5$	9,10,11-trihydroxy-12-octadecenoic acid
6.060	312.2533 $[M + H]^+$	312.2533			$C_{18}H_{33}NO_3$	unknown
6.458	885.4842 $[M + H]^+$	885.4842	929.4747 $[M + HCOO]^-$	929.4752	$C_{45}H_{72}O_{17}$	gracillin
6.524	315.2537 $[M + H]^+$	315.2530	313.2383 $[M - H]^-$	313.2384	$C_{18}H_{34}O_4$	lipid derivative

Table 8. *Cont.*

Positive Ion Mode			Negative Ion Mode		Molecular Formula	Metabolite Name
RT	Found	Calcd	Found	Calcd		
6.805	415.2117 $[M + H]^+$	415.2115			$C_{24}H_{30}O_6$	bersenogenin
6.899	478.2943 $[M + H]^+$	478.2952	476.2794 $[M - H]^-$	476.2806	$C_{30}H_{39}NO_4$	18-deoxycytochalasin H
7.044	339.2509 $[M + Na]^+$	339.2506	315.2541 $[M - H]^-$	315.2541	$C_{18}H_{36}O_4$	lipid derivative
7.086	310.2381 $[M + H]^+$	310.2377	308.2232 $[M - H]^-$	308.2231	$C_{18}H_{31}NO_3$	lipid amide
7.296	492.1134 $[M + NH_4]^+$	492.1137	473.0724 $[M - H]^-$	473.0725	$C_{22}H_{18}O_{12}$	cichoric acid
7.539	891.4708 $[M + Na]^+$	891.4713	913.4790 $[M+HCOO]^-$	913.4802	$C_{45}H_{72}O_{16}$	dioscin
7.657	280.2640 $[M + H]^+$	280.2635			$C_{18}H_{33}NO$	linoleamide
7.672	449.3732 $[M + Na]^+$	449.3757			$C_{30}H_{50}O$	amyrin
7.782			295.2280 $[M - H]^-$	295.2279	$C_{18}H_{32}O_3$	9-hydroxy-10,12-octadecadienoic acid
7.887	257.2478 $[M + H]^+$	257.2475	255.2329 $[M - H]^-$	255.2330	$C_{16}H_{32}O_2$	palmitic acid
7.970			358.2595 $[M - H]^-$	358.2599	$C_{19}H_{37}NO_5$	unknown
7.990	296.2592 $[M + H]^+$	296.2584			$C_{18}H_{33}NO_2$	stearimide
8.076	437.3729 $[M + Na]^+$	437.3754			$C_{29}H_{50}O$	*β*-sitosterol
8.235	685.4366 $[M + Na]^+$	685.4356			$C_{42}H_{63}O_4P$	tris-(2,4-di-*tert*-butylphenyl)phosphate
8.251	239.2377 $[M + H]^+$	239.2397			$C_{16}H_{30}O$	2-hexadecenal
8.248	281.2481 $[M + H]^+$	281.2475	279.2327 $[M - H]^-$	279.2330	$C_{18}H_{32}O_2$	linoleic acid
8.400	453.3685 $[M + Na]^+$	453.3703			$C_{29}H_{50}O_2$	*α*-tocopherol
8.704	228.2329 $[M + H]^+$	228.2322			$C_{14}H_{29}NO$	myristamide
9.025	254.2484 $[M + H]^+$	254.2478			$C_{16}H_{31}NO$	palmitoleamide
9.550			271.2277 $[M - H]^-$	271.2279	$C_{16}H_{32}O_3$	unknown
9.689	307.2628 $[M + H]^+$	307.2632	351.2539 $[M+HCOO]^-$	351.2541	$C_{20}H_{34}O_2$	ethyl linolenate
9.868	256.2638 $[M + H]^+$	256.2625			$C_{16}H_{33}NO$	palmitamide
10.109	282.2791 $[M + H]^+$	282.2791			$C_{18}H_{35}NO$	9-octadecenamide
10.147	309.2787 $[M + H]^+$	309.2788	353.2694 $[M + HCOO]^-$	353.2697	$C_{20}H_{36}O_2$	ethyl linoleate
11.58	285.2794 $[M + H]^+$	285.2788	283.2643 $[M - H]^-$	283.2643	$C_{18}H_{36}O_2$	ethyl palmitate

3.1. Chemical Composition by GC-MS Analyses in Various Fractions of Dioscorea communis Berry Juice

At first, the chemical composition of the berry juice was achieved by two approaches. Extracts with different polarities were obtained through liquid-liquid extraction (see Section 2.3 and Figures S1–S18). The untreated (A1A and A1B) and after acid hydrolysis non-polar extracts (A2A and A2B) were submitted to GC-MS analyses, revealing the presence of several fatty acid esters (Tables 1–4). It is noteworthy that both qualitative and quantitative differences were observed after acid hydrolysis, which could be attributed to the hydrolysis of fatty acid esters, triglycerides, or phospholipids [38]. The chemical fingerprints of the untreated Et_2O (A1A) and CH_2Cl_2 (A1B) extracts were quite similar, with ethyl linoleate (45.2% and 46.7%, respectively) and ethyl linolenate (36.9% and 38.1%, respectively) being their main metabolites. After acid hydrolysis, the obtained Et_2O extract (A2A) was characterized by the presence of methyl esters of palmitic (53.3%), linoleic (20.3%), and linolenic (14.8%) acid, while the CH_2Cl_2 extract (A2B) was abundant in methyl palmitate (33.2%), ethyl linoleate (28.1%), and ethyl linolenate (24.2%). It was noticed that the ethyl esters of linoleic and linolenic acid were present in all extracts, while methyl linolenate was absent in the untreated Et_2O extract (A1A). Phthalates, such as dibutyl phthalate (Table 1, compound **1**), are used as plasticizer solvents. Thus far, they have been previously described from the genus *Dioscorea* and the family Dioscoreaceae, as well as from other natural sources [39]. However, their presence as natural products is controversial, as they could be either stored from the environment or co-extracted using solvents during the handling of the plant material [40].

For a more detailed analysis, part of the lyophilized berry juice was subjected to RP_{18}-MPLC, and the yielded fractions were screened by ^{1}H-NMR. Based on the obtained spectra, three fractions (AI, AE, and AG) were selected and further analyzed by GC-MS (Tables 5–7). Briefly, the fractions AI, AE, and AG were mixtures of fatty acid esters, ketones, aldehydes, alcohols, and hydrocarbons. In detail, 23 compounds were detected in fraction AI, with 2-octadecanone (34.0%) and 2*E*-nonadecene (9.4%) being the main constituents.

In fraction AE, 10 compounds were detected, and once more the main ingredient proved to be 2-octadecanone (49.4%), while methyl stearate was also abundant (20.8%). Fraction AG featured 19 compounds, and again the predominant compound was 2-octadecanone (59.7%), followed by methyl stearate (11.5%) and an unknown compound (11.6%), with m/z = 313.3. The available GC-MS libraries do not include data regarding *N*- and *P*-containing compounds. However, the odd m/z values suggested the presence of such compounds, which was further supported by the LC-MS analysis.

3.2. NMR Analyses in Non-Polar Fractions of Dioscorea communis Berry Juice

The NMR analyses of all non-polar extracts (A1A, A1B, A2A, and A2B) confirmed the presence of fatty acid esters. The olefinic protons (–C<u>H</u>=C<u>H</u>–) of the unsaturated fatty esters appeared as multiplets at δ_H ca.5.34. The methyl group of the methyl esters (–OCOC<u>H</u>$_3$) appeared as singlet at δ_H 3.65, while the terminal methyl group of the alkyl chain appeared as triplets at δ_H ca. 0.88 or 0.95, depending on the degree of unsaturation. Moreover, the terminal methylene group of ethyl esters (–OCOC<u>H</u>$_2$CH$_3$) appeared as quadruplets at δ_H ca. 4.12 ($J \approx 6.9$). The vicinal methylene of the esters (–C<u>H</u>$_2$COOR) resonated at δ_H ca. 2.33 (t, $J \approx 6.8$), while the methylene (–C<u>H</u>$_2$–) between the double bonds of the unsaturated fatty esters resonated at δ_H ca. 2.76 (m). The intense signal at δ_H ca. 1.24 was assigned to the rest of the methylenes of the alkyl chains, partially overlapping the triplet of the terminal methyl group (–OCOCH$_2$C<u>H</u>$_3$) of the ethyl esters (Figure 1). Similarly, corresponding signals for the ketones are depicted in Figure 2. In the case of triacylglycerol esters, the peak of the proton at C-2 of glycerol appeared at δ_H ca. 5.30 as a triplet of triplets, while the methylenes of positions C-1 and C-3 of glycerol resonated at δ_H ca. 4.33 (q) and 4.17 (q) (Figure 2).

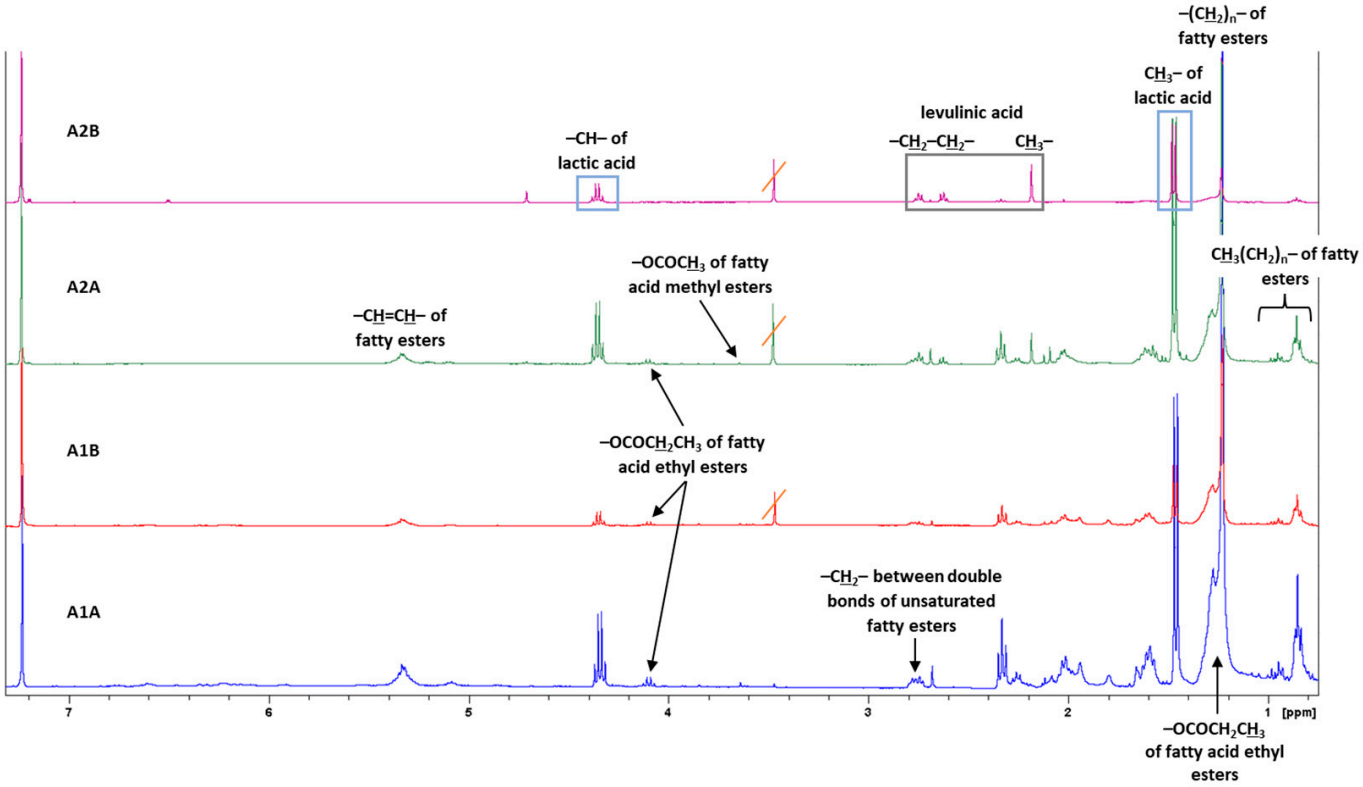

Figure 1. ^{1}H-NMR spectra ($CDCl_3$, 400 MHz) of non-polar extracts from *Dioscorea communis*. A1A = ether extract; A1B = dichloromethane extract; A2A = ether extract after acid hydrolysis; and A2B = dichloromethane extract after acid hydrolysis.

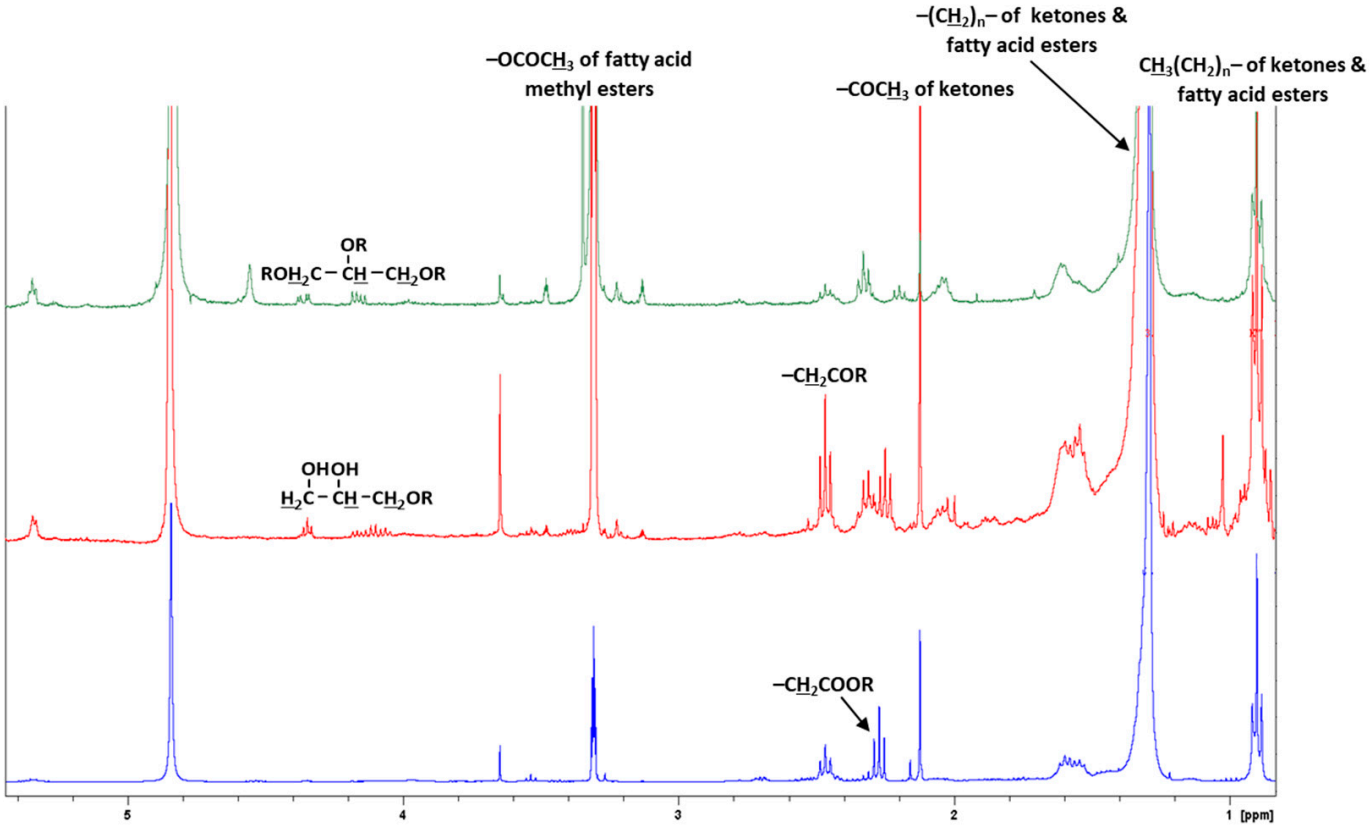

Figure 2. ^{1}H-NMR spectra (CD_3OD, 400 MHz) of the fractions AE, AG, and AI.

In agreement with the GC-MS analyses, both Et_2O extracts (A1A and A2A) were mainly characterized by the presence of ethyl esters. In addition, the Et_2O extract after acid hydrolysis (A2A) revealed the presence of methyl esters, while the CH_2Cl_2 extract (A2B) consisted of methyl and ethyl esters. It is worth mentioning that the CH_2Cl_2 extract after acid hydrolysis (A2B) was remarkably different, since the fatty acid esters and unsaturated derivatives were minor metabolites. The main compounds of this extract were lactic (**1**) and levulinic acids (**2**) not detected through the GC-MS analyses. Concerning the polar *n*-butanol extract (A3), the main signals of the ^{1}H-NMR spectrum were assigned to lactic acid (Figure S33).

3.3. LC-MS/MS Analysis of n-Butanol Extract of Dioscorea communis Berry Juice

The *n*-butanol extract (A3) of the berry juice obtained after liquid-liquid extraction (Section 2.3) was submitted to LC-MS/MS analysis. The putative identification of these compounds is summarized in Table 8, where the compounds are listed according to their retention times in the total ion chromatogram (TIC) (Figures S34 and S37). Its ^{1}H-NMR spectrum was also measured (Figure S33). Based on these results, the main constituent of A3 was lactic acid. Moreover, more than 45 compounds were tentatively identified by LC-MS analysis, including amino acids, organic acids, sugars, fatty acid derivatives, *N*-containing derivatives, flavonoids, phenolic acids, and other phenolic derivatives. The molecular formulas were established based on high-precision quasi-molecular ions such as $[M - H]^-$, $[M + CH_3COO]^-$, $[M + HCOO]^-$, $[M + H]^+$, or $[M + Na]^+$ with a mass error of 5.0 ppm, and all information was interpreted and compared with the spectra available in the literature. More specifically, the LC-MS results revealed that the berry juice extract contained various carbohydrates, including mono- and di-saccharides ($C_6H_{12}O_6$ 203.0530 $[M + Na]^+$, *m*/*z*, $C_{12}H_{22}O_{10}$ 349.1121 $[M + Na]^+$, and $C_{12}H_{22}O_{11}$ 365.1059 $[M + Na]^+$), amino acids like phenylalanine ($C_9H_{11}NO_2$ 166.0866 $[M + H]^+$), simple organic acids such as ascorbic acid, fumaric acid ($C_6H_8O_6$ 175.0250 $[M - H]^-$ and $C_4H_4O_4$ 117.0182 $[M + H]^+$), a variety of

phenolic derivatives including caffeic and cichoric acids, kaempferol glycosides, quercetin and its glycosides (e.g., $C_{27}H_{30}O_{15}$ 539.1516 $[M - H]^-$ and $C_{15}H_{10}O_7$ 301.0353 $[M - H]^-$), as well as tris-(2,4-di-*tert*-butylphenyl)phosphate ($C_{42}H_{63}O_4P$ 685.4366 $[M + Na]^+$), with numerous fatty acid derivatives (Table 8). The chemical evaluation was in agreement with previous reports on the genus *Dioscorea* and the Dioscoreaceae family.

It is noteworthy that the accumulation of fatty acid derivatives and phytosterols is essential during fruit development and ripening. For example, stearic acid, although more abundant in animals, can also be found in vegetable fat. Linolenic acid is mostly found in seeds and berries, while ethyl palmitate is among the most common saturated fatty acid esters in plants [41]. Other commonly detected metabolites in plant extracts, like phytosterols (β-sitosterol) and triterpenes (amyrin), also have physiological roles in plants. For example, phytosterols are naturally present in plant cell membranes, and triterpenes are associated with plant defense [42]. Furthermore, *N*-alkylamides are essential for plant immunity, usually being produced as a response to abiotic (non-pathogen-induced) and biotic (pathogen-induced) stress. Such compounds act as a chemical defense against phytopathogens and herbivorous predators. Many pathways lead to the expression of defense-related genes, including the production of anti-microbial secondary metabolites like alkylamides [43]. The monitoring of amides in LC-MS was found to be more effectively performed in a positive mode where the carboxamide group is protonated. However, both positive and negative ionization modes were used in the current study, as the negative mode was reported to be more sensitive in the analysis of phenolics and other compounds [44].

3.4. Isolated Compounds of Dioscorea communis Berry Juice

Finally, the lyophilized berry juice yielded (Section 2.3) lactic acid (**1**) [45], levulinic acid (**2**) [46], 2-octadecanone (**3**) [47], and two phenolic compounds: the rare tris-(2,4-di-*tert*-butylphenyl)phosphate (**4**), previously isolated from *Vitex negundo* [48], as well as cichoric acid (**5**) [49].

3.5. Antibacterial Activity of Dioscorea communis Berry Juice and Selected Fractions

To the best of our knowledge, *D. communis* berry juice has been assessed regarding its antibacterial activity for the first time. Thus far, previous studies on *D. pentaphylla* and *D. bulbifera* extracts and fractions from different plant parts revealed their antibacterial activity [21,50,51].

In the present study, *D. communis* berry juice exhibited bactericidal activity against MRSA and *C. acnes*. Its MIC and MBC values were determined to be 1.56% w/v against both bacteria. These results indicate that berry juice might be considered a novel source of antibacterial substances against these two bacteria, which are often implicated in dermatological infections and acne. Moreover, the fraction AD exhibited bacteriostatic activity against *C. acnes*, with an MIC at 6.6 mg/mL. Based on our chemical analyses, the effect could be attributed to 2-octadecanone (compound **3**), methyl stearate, and tris-(2,4-di-tert-butylphenyl)phosphate (compound **4**), which were identified in fraction AD (Figures S12–S18 and Table S1).

4. Conclusions

In this study, GC-MS analysis offered influence measurements on the non- and less-polar components with a key role in the characterization of 22 fatty acid derivatives. On the other hand, LC-MS analysis comprises a wide variety of compounds predominant as primary or secondary metabolites, such as amino acids (1), organic acids (3), lipids (14), terpenes-sterols (6), sugars, and phenolics (8), and NMR offers the structure elucidation of 5 individual components, as well as the metabolite fingerprinting. These methods were equally adapted in order to provide both an inclusive impression and complete analysis of the critical components existing in the plant material. The antibacterial activity of *D. communis* berry juice against pathogens often implicated in dermatological infections has been reported herein for the first time. MRSA and *C. acnes* were used, showing MIC

and MBC values at 1.56% *w/v* against both bacteria, which warrants further investigation as this may lead to medical applications. It is notable that these bacteria are resistant to several antibiotics, and treatments that target multiple pathological processes of skin abnormalities are accompanied by side effects [52,53]. Therefore, alternative therapies are urgently needed. Nevertheless, future studies for the evaluation of the acute and sub-acute toxicity effects of the berry juice extract should be conducted.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/sci4020021/s1. Figures S1–S40: GC-MS chromatograms of the fractions A1A, A1B, A2A, A2B, AE, AG, AI, AD_B, and AF_A; Spectra of the known compounds **1–5**; LC-MS chromatograms of n-butanol extract (A3) in positive and negative ion mode; LC-MS data of selected compounds in positive and negative ion mode; Flow chart of the isolation procedures; GC-MS tables of AD_B and AF_A. Table S1: Chemical composition of fraction AD_B. Table S2: Chemical composition of fraction AF_A.

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