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Proximate analysis and GC-MS phytochemical profiling of aqueous extracts of *Doryopteris raddiana*, a plant used by the Mbya-Guaraní as a contraceptive

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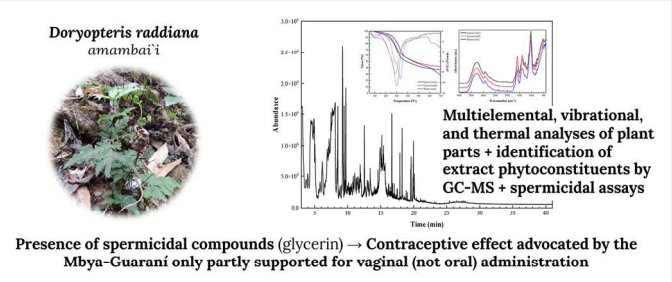
ABSTRACT

Doryopteris raddiana (Presl) Fée, a traditional contraceptive in Mbya culture, lacks scientific scrutiny regarding its chemical composition and contraceptive efficacy. Employing X-ray fluorescence, Fourier-transform infrared spectroscopy, and thermal analysis, we explored the plant's organs. Multielemental analysis excluded toxic elements. Key phytoconstituents identified by gas chromatography-mass spectrometry in the extracts obtained through infusion were glycerine, 1,3-dimethyl propane, and catechol in leaves; glycerine, cis-13-octadecenoic acid methyl ester, and 2-deoxy-D-erythro-pentose in stems and roots. Among these chemicals, glycerine emerged as the sole constituent with contraceptive potential, particularly intravaginally. Extract activity tests conducted on ram spermatozoa exhibited a reduction in the percentage of rapid spermatozoa but no significant impact on total motility, progressive motility, or viability. The reported data would only weakly support the advocated contraceptive action of this fern upon vaginal application, not through the oral administration of its decoction.



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1. Introduction

Historically, the Guaraní occupy the lowland region of South America, mainly in Paraguay, Uruguay, and Parana river basins, and on the southern Atlantic coast of Brazil. The Guaraní presence in the region is estimated to date back ca. 2000 years. The Guaraní are currently divided into three main groups: Mbya Guaraní, Ava Guaraní/Ñandéva, and Paĩ Tavyterã/Kaiowá. According to Martínez Crovetto (1968) and Votre et al. (2017), the success of Guaraní occupations in forest environments is due to their botanical and ecological knowledge of medicinal and healing plants (using the term 'healing' to encompass magic (*ka'avo*) and ritualistic plants, given that medicine and spirituality are intertwined in Guarani worldview) (Oliveira 2009; Valdez et al. 2015).

Martínez Crovetto (1968) cited the use of 438 plants among Mbya. One of the most appreciated was the fern *Doryopteris raddiana* (C. Presl) Fée (syn. *Hemionitis raddiana* (C.Presl) Christenh.), a *Pteridaceae* (Peña-Chocarro et al. 1999). The term used by the Guaraní to designate *D. raddiana* is *amambaìi*.

According to an ethnographic study carried out with funding from the National Council of Science and Technology of Paraguay (Fogel Pedroso et al. 2016) that covered the properties of 86 medicinal plants used in six Mbya Guaraní indigenous communities, women use this fern as a contraceptive.

Its administration can be done in a vaginal way or by drinking a decoction of the aerial parts of the plant or its entirety. Curiously, the only mode of administration referred to by the informant Guaraní women interviewed in the present study was the oral way (documented in audiovisual material available at the Interdisciplinary Rural Studies Centre (Asunción, Paraguay) repository, in which a Mbya Guaraní woman shows the collection of the plant from its natural habitat and its preparation). The primarily suggested preparation and dosing regimen was [tr.]:

> Boil a handful of the plant's leaves in a litre of water for ten minutes. Drink a quarter litre of this decoction during menstruation, starting on the third day and ending on the 22nd day. Repeat this process in the following month. Avoid sexual intercourse while undergoing this treatment. After it is complete, the woman will no longer be able to have children.

A different alternative was as follows [tr.]:

> The entire plant should be boiled for ten minutes in a litre of water. A quarter litre of the decoction should be taken twice a day, in the morning and the afternoon; it can also be consumed in a mate [a traditional South American infused prepared with dried leaves of *Ilex paraguariensis* A.St.-Hil.]. The duration of treatment depends on the number of days of menstruation. The decoction should be taken starting from the first day in which menstruation ceases and for a period of time equal to the length of menstruation (i.e., if menstruation lasts four days, the decoction should be taken for four days). The process should be repeated for five months to avoid having children again.

Given that, to the best of the authors' knowledge, a phytochemical study of this plant has not been conducted to date, the aim of the study presented herein is two-fold: to investigate the constituents present in the plant and its extracts and to determine if the presumed contraceptive activity is backed up by scientific evidence. In addition, given that the Convention of Biological Diversity's Nagoya protocol (Moody 2020) establishes that the patenting of this traditional knowledge by third parties requires novelty (which implies that there have been no previous publications on the properties

of the plants in question and their preparations), this study can be useful to preserve the rights of the Mbya Guaraní on this plant and its preparations for medicinal use.

2. Results and discussion

2.1. Multielement analysis and ash contents

Results from X-ray fluorescence spectroscopy (XRF) characterisation of plant components and ashes (Table S1) revealed that, while roots accumulate Fe, Mn, Ti, and Al, leaves and stems store high contents of Zn, Mg, K, Cl, and Si. Upon heating up to 900 °C, the ash content of leaves was slightly lower than that of stems and roots.

Analysis of the mineral elements in the samples ruled out the presence of toxic elements or non-toxic elements with elevated levels that could indicate the occurrence of contaminated soils or an abnormal accumulation that could account for the contraceptive effects.

2.2. Extract characterization by GC–MS

From the gas chromatography-mass spectrometry (GC–MS) chromatograms registered for leaf and stem and root extracts of *D. raddiana* (Figures S4 and S5), the identified phytochemical constituents are summarised in Tables S2 and S3 for leaves and stems and roots, respectively.

In the extracts of *D. raddiana* leaves, the most abundant compounds were glycerine (28.3%); 1,3-dimethyl-propane (6.0%); ethylhydrazine (4%); catechol, CTC (4.1%); heptanoic acid (2.4%); 5-hydroxymethylfurfural, 5-HMF (2.2%); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, DDMP (2.1%). Other phytochemicals present in the leaf extracts that may influence the reproductive function include 9,12,15-octadecatrienoic acid (*α*-linolenic acid) and its methyl ester (1.5%); N-methoxy methyl-N-methyl acetamide (1.1%); and N-methyl-N-nitroso-2-propanamine, NMIPA (0.7%).

In turn, the main phytochemicals identified in the stem and root extract were glycerine (20%); *cis*-13-octadecenoic acid, methyl ester (9.3%); 2-deoxy-D-erythro-pentose (or deoxyribose) (6.9%); methyl-D-glucopyranoside (6.5%); 1,3-dihydroxyacetone (6.3%); 2-hydroxy-2-cyclopenten-1-one (5.4%); 1,3,5-triazine-2,4,6-triamine (3.6%); DDMP (2.6%); diglycerol (2.0%), methyl *α*-D-xylofuranoside (1.8%); and 1-methyl-2-pyrrolidinone, NMP (0.5%).

It is worth noting that the methyl esters may be associated with the use of methanol as a solvent for the GC–MS analyses (Venditti 2018), provided that it can function as a derivatizing agent for some polar compounds. However, given that in this study derivatization was not performed to improve the detectability of certain compounds, especially non-volatile and polar ones, a word of caution seems necessary, as the reported profiling may not be comprehensive.

2.3. Spermicidal activity tests

No significant differences in sperm total or progressive motility or viability were observed in samples incubated with the plant lyophilizate or extract throughout the

incubation time. However, the percentage of rapid spermatozoa was significantly lower in the samples with the plant extract ($p < 0.05$) from the second hour of incubation (Table S4).

2.4. On the reported activity of some identified constituents

Glycerine, apart from being an antispermatogenic agent (high glycerine concentrations promote the apoptosis of germ-line cells, which causes temporary spermatogenesis arrest, and may also result in permanent oligospermia or even azoospermia (Crisóstomo et al. 2017)), would be the only constituent with a clear effect as a spermicide. Even though it is used as a cryoprotective agent to prevent injury to human spermatozoa during the cryopreservation process (at ca. 1 M concentrations) (Gao et al. 1995), it is toxic to spermatozoa. This is due in part to the osmotic stress that it imposes on the cell, but also because glycerine has direct effects on the structure of the plasma membrane and the metabolism of the cell (Hammerstedt et al. 1990). Concentrations of glycerine of 2% in vaginal lubricants have been reported to significantly impair sperm motility and progression (Tulandi and McInnes 1984), with a decreased ability of sperm to fertilise and support embryo development attributed to sublethal damage (Wright 2010). Nonetheless, as the assayed concentration, which was at least ten times lower than the above referred one (considering a 6 mg·mL^{-1} extract concentration and a 20–27% glycerine content), only a statistically significant decrease in the number of rapid spermatozoa was observed.

Concerning other phytochemicals present in the extracts, they may influence male reproductive function, but would not be relevant in terms of the contraceptive effect of the fern extract, given that it is consumed by the Mbya women. In particular, α-linolenic acid has been evidenced to be antiandrogenic, showing a proliferation inhibitory effect on lymph-node carcinoma of the prostate cells (Liu et al. 2009). Ethylhydrazine suffers dehydrogenation to form a diazene and is responsible for the induction of unscheduled DNA synthesis in the germ cells of male mice (Sotomayor et al. 1982). N-methoxymethyl-N-methylacetamide is related to N,N-dimethylacetamide, an effective male contraceptive agent (Khera et al. 2020), and pesticide Alachlor/Pestanal™ or 2-chloro-N-(2,6-diethyl)phenyl-N-methoxymethylacetamide, with adverse effects on human sperm motion (Grizard et al. 2007). Finally, as regards NMP (whose reproductive toxicity and gonadotoxic potential in male rats was studied by Sitarek and Stetkiewicz (2008) and Khera et al. (2021)), it has been demonstrated that exposure to this bromodomain inhibitor blocks spermatogenesis in a hormonal and non-hormonal fashion.

2.5. On the presumed efficacy of 'amambai`i' medicine

Regarding the mode of administration of this medicinal fern, the preferred way was orally, either by decoction, maceration, or infusion. Nevertheless, the advocated contraceptive effect of *D. raddiana*, attributed by Guarani women, cannot be achieved in this way, at least using this plant alone. None of the components identified in the phytochemical profile of this fern could support the sough application using the oral

route as an administration mode. Taking into consideration that one of *D. raddiana* phytochemicals can function as a spermicide, which must be placed deep in the vagina (close to the cervix) 10–15 min before sex to be effective, effective application of *amambai'i* by intake only could be achieved by combining it with other drugs (to date, unidentified) that could confer contraceptive properties to the mixture.

From the statements of some of the informants [tr.], the inefficiency of the declared mode of current consumption seems to be inherently recognised:

> We use the *amambai'i* plant alone, for now, because we no longer have the other plants to combine it with. We use it for a longer time so that it has the expected contraceptive effect.

> Since I had my first child, to space out pregnancies, I take care of myself with medicinal plants. If I can't find them in my community, I go to another to get them. We combine this *amambai'i* plant with two other plants, which at present are difficult to obtain.

> My grandmother used *amambai'i* combined with other plants, and she also prepared it for other women. We continue that tradition, but with difficulties in accessing the other plants […], so we consume the plant alone and for longer periods.

It is highly suggestive that *D. raddiana* does not appear in the compilation by Basualdo et al. (2004), in the list of medicinal plants used in Primary Health Care in Paraguay (Soria and Ramos 2015), nor in the recent article by Kujawska and Schmeda-Hirschmann (2022) on medicinal plants based in the Guaraní tradition.

In a critical analysis, a possible limitation of the study has been identified concerning the validity of the information obtained in the public survey. The authors suspect that the responses from the informants may not be complete or entirely truthful, given that they are strongly sensitised by opinion campaigns about the theft of traditional knowledge for commercial gain (Moody 2020).

2.6. On other potential valorisation pathways

Other potential uses of *D. raddiana* extracts may be explored based on the identified phytochemicals. For example, five of the most abundant components in the extract, such as *cis*-13-octadecenoic acid methyl ester, catechol, 2-deoxy-D-erythro-pentose, 5-hydroxymethyl formamide, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, have been demonstrated to be effective as food preservatives (Ramalakshmi and Muthuchelian 2011; Kant et al. 2019) and for the control of pathogens (AlAmery and AlGaraawi 2020). In particular, *cis*-13-octadecenoic acid has been shown to possess antibacterial action against *Bacillus cereus* and *Escherichia coli* (Ahmad et al. 2020); catechol has been reported to inhibit bacterial phytopathogens such as *Clavibacter michiganensis* subsp. *michiganensis* and *Xanthomonas campestris* pv. *vesicatoria* (MIC = 375 and 250 ug·mL^{-1}, respectively); 5-HMF has been active against human bacterial pathogens such as *Klebsiella* spp. (MIC = 40–160 µg·mL^{-1}) (Kaur et al. 2018) and against phytopathogens such as *Erwinia amylovora*, *Xylophilus ampelinus,* and *Diplodia seriata* (Sánchez-Hernández et al. 2022); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one demonstrated activity against plant pathogens such as *Phytophthora megasperma* (375 ug·mL^{-1}), *Verticillium dahliae* (375 ug·mL^{-1}) and *Diaporthe amygdali* (750 ug·mL^{-1}) (Sánchez-Hernández et al. 2023).

3. Conclusion

Gas chromatography-mass spectroscopy analytical findings do not support the purported contraceptive efficacy of orally ingested *D. raddiana* in the form of a decoction, maceration, or infusion. Glycerine, present in the extracts at a concentration of between 20 and 27%, was the only constituent that could potentially have a contraceptive effect if applied intravaginally due to its toxicity to spermatozoa. Nonetheless, at the 6 mg·mL^{-1} extract concentration that was assayed on Rasa Aragonesa rams' spermatozoa, only a decrease in the percentage of rapid spermatozoa was observed, without statistically significant differences in total or progressive motility or viability of the sperm. Thus, the reported data only partially verify the suggested contraceptive action of this fern upon vaginal application and suggest that *amambai'i* cannot be considered a reliable herb for oral contraception.

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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Abstract: *Doryopteris raddiana* (Presl) Fée, a traditional contraceptive in Mbya culture, lacks scientific scrutiny regarding its chemical composition and contraceptive efficacy. Employing X-ray fluorescence, Fourier-transform infrared spectroscopy, and thermal analysis, we explored the plant's organs. Multielemental analysis excluded toxic elements. Key phytoconstituents identified by gas chromatography-mass spectrometry in the extracts obtained through infusion were glycerine, 1,3-dimethyl propane, and catechol in leaves; glycerine, cis-13-octadecenoic acid methyl ester, and 2-deoxy-D-erythro-pentose in stems and roots. Among these chemicals, glycerine emerged as the sole constituent with contraceptive potential, particularly intravaginally. Extract activity tests conducted on ram spermatozoa exhibited a reduction in the percentage of rapid spermatozoa but no significant impact on total motility, progressive motility, or viability. The reported data would only weakly support the advocated contraceptive action of this fern upon vaginal application, not through the oral administration of its decoction.

SUPPLEMENTARY MATERIAL

1. EXPERIMENTAL

1.1. Samples

Amambai`i fern samples under study were collected by two of the co-authors with the guidance of the Guavirami Trinidad indigenous community of the Department of Itapúa of the Eastern Region of Paraguay (27°10′S 55°75´W), as shown in Figure S1. Voucher specimens were deposited at the Herbarium of the Faculty of Chemical Sciences of the National University of Asunción (Asunción, Paraguay), with voucher specimen number 'Mitapokaja CC1615'.

Figure S1. Collection of the contraceptive fern *Doryopteris raddiana* (‘amambái’, ‘amambai`i’, or ‘amambai´i’) by one of the co-authors.

1.2. Extract Preparation

Plant organs from different specimens (n = 20) were thoroughly mixed to obtain (separate) leaf composite samples and stems and roots composite samples, due to the discrepancies between recipes that specified either only leaves or the entire plant. The composite samples were shade-dried, pulverized to a fine powder in a mechanical grinder, homogenized, and sieved (1 mm mesh).

The obtaining of *D. raddiana* extracts was conducted according to the traditional recipe described in the introduction section (i.e., by a water decoction), with slight modifications to replicate standard procedures defined for ‘basic substances’ under the EU plant protection products regulation (Article 23 of (EC) No 1107/2009), such as the procedure described in Appendix I in SANCO/12386/2013 for *Equisetum arvense* L. In short, 20 g of the dry plant was macerated in 1 L of water for 30 min (soaking) and then boiled for 10 min. After cooling down, the decoction was filtrated and further diluted with water, to obtain a final concentration of 6 $mg \cdot mL^{-1}$. Part of the solution was freeze-dried to get the solid residue. 25 mg of the obtained freeze-dried aqueous extract were then dissolved in 5 mL of HPLC grade methanol to get a 5 $mg \cdot mL^{-1}$ solution, which was filtered and used for GC–MS analysis.

1.3. Characterization Procedures

X-ray fluorescence spectroscopy was used to determine the multielement composition of the samples, using a Niton XL3t GOLDD+ portable analyser (Thermo Fisher Scientific, Waltham, MA, USA). X-ray tube: Au anode, 50 kV, 200 μA; spot size: 8 mm; 3 mm small-spot collimation. Soil (traces) mode was used, with at least 3 measurements per analysis, with a 240 s acquisition time/measurement.

Ash contents were determined by combustion at 900 °C, according to the TAPPI T 413 om-17 method (Technical Association of the Pulp and Paper Industry, 2016).

The infrared vibrational spectra were registered using a Nicolet iS50 Fourier-transform infrared spectrometer (Thermo Scientific; Waltham, MA, USA) with a diamond attenuated total reflectance (ATR) system. The spectra were collected over the 400–4000 cm^{-1} range, with a 1 cm^{-1} spectral resolution, and the interferograms that resulted from co-adding 64 scans were taken.

The extracts of leaves and stems and roots were analysed by gas chromatography-mass spectrometry using a gas chromatograph model 7890A coupled to a quadrupole mass spectrometer model 5975C (both from Agilent Technologies; Santa Clara, CA, USA) at the Research Support Services (STI) at Universidad de Alicante (Alicante, Spain). Chromatographic conditions included an injection volume of 1 µL, an injector temperature of 280 °C in splitless mode, and an initial oven temperature of 60 °C for 2 minutes, followed by a ramp of 10 °C·min^{-1} up to a final temperature of 300 °C for 15 minutes. The chromatographic column used for the separation of the compounds was an Agilent Technologies HP-5MS UI of 30 m in length, 0.250 mm diameter, and 0.25 µm film. Mass spectrometer conditions included a temperature of the electron impact source of the mass spectrometer of 230 °C, a temperature of the quadrupole of 150 °C, and an ionization energy of 70 eV. The identification of extract constituents was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with the database of the National Institute of Standards and Technology (NIST11) and Adams (2007).

The thermogravimetric/derivative thermogravimetric analyses of the samples were conducted with a STA6000 simultaneous thermal analyser (Perkin-Elmer; Waltham, MA, USA) by heating the samples in a slow stream of N_2 (20 mL·min^{-1}) from room temperature up to 700 °C, at a heating rate of 20 °C·min^{-1}. The data analysis was performed using the Perkin Elmer Pyris v.11 software.

1.4. Spermicidal Activity Assays

1.4.1. Experimental Design

To test the effect of the plant lyophilizate and extract on spermatozoa, the lyophilizate (30 mg) was dissolved with 5 mL of phosphate-buffered saline (PBS), leaving a concentration of 6 mg·mL^{-1}, and the extract was used as prepared (6 mg·mL^{-1}), according to the procedure detailed in subsection 4.2.

Ram ejaculate was diluted 1/100 (3×10^7 cells·mL^{-1}) in a suitable medium (0.25 M sucrose, 100 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 0.5 mM sodium phosphate, 50 mM glucose, 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 20 mM KOH) and divided in aliquots: *L* (plus 100 µL of the reconstituted lyophilizate) and *E* (plus 100 µL of the extract). Moreover, two aliquots were added as controls: C_L (plus 100 µL of PBS) and C_E (plus 100 µL of bi-distilled water).

Samples were analysed from the moment of addition of the compound (t=0) and every hour of incubation at 37 °C. Total motility, progressive motility, percentage of rapid spermatozoa and viability (plasma membrane integrity) were evaluated as described below. Experiments were conducted in triplicate and results were expressed as mean ± SEM (n=3).

1.4.2. Semen Collection

Semen from nine Rasa Aragonesa rams (2–4 years old) was collected with the aid of an artificial vagina. All the rams belonged to the Rasa Aragonesa National Breeding Association (ANGRA) and were kept at the Experimental Farm of the Veterinary School of the University of Zaragoza under the same nutritional conditions. All experimental procedures were performed under the project license PI39/17 approved by the University of Zaragoza Ethics Committee for Animal Experiments (approval date: 24 May 2017), following the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Two successive ejaculates were collected every two days, and second ejaculates were pooled and processed together to avoid individual differences. Ejaculates were kept at 37 °C until analysis.

1.4.3. Sperm Motility Analysis

A computer-assisted sperm analysis system (CASA) was used for analysing sperm motility (ISAS v. 1.04, Proiser S.L., Valencia, Spain). A drop of 8 µL of each aliquot was placed between a pre-warmed slide and a

coverslip and maintained at 37 °C in a heated slide holder during analysis. Spermatozoa were recorded using a video camera (Basler A312f, Basler Vision Components, Exton, Pennsylvania, USA) mounted on a microscope (Nikon Eclipse 50i, Nikon Instruments Int, Tokyo, Japan) equipped with a 10× negative-phase contrast lens. The recording was performed at 25 frames·s^{-1} and 25 consecutive digitalized images were taken for a single field. Five fields of each drop were recorded, and percentages of total motile (% of sperm with moving), progressive motile (% of spermatozoa with progressive movement), and rapid (velocity >75 μm·s^{-1}) spermatozoa in all samples were evaluated.

1.4.4. Evaluation of Sperm Membrane Integrity

To determine cell membrane integrity (viability), 3 μL of 10 μM carboxyfluorescein diacetate (CFDA), 3 μL of 7.3 μM propidium iodide (PI), and 3 μL formaldehyde (0.5 % (*v/v*) in water) were added to 500 μL of sperm samples (final concentration 5×10^6 cells·mL^{-1}), based on a modification of the procedure described by Harrison and Vickers (1990). Samples were incubated at 37 °C in darkness for 15 min and evaluated by flow cytometry using a Beckman Coulter FC 500 flow cytometer (Beckman Coulter Inc, Fullerton, California, USA). A minimum of 20,000 events were counted in all experiments. The sperm population was identified for further analysis by the specific forward (SF) and side scatter (SS) properties; thus, an SF-SS plot was used to exclude non-sperm particles from the analyses. The monitored parameters were SF log, SS log, FL1 log (CFDA), and FL4 log (PI). The percentages of membrane intact cells (viable spermatozoa) were considered.

1.5. Statistical Analyses

Differences between samples were analysed by a mixed model ANOVA and Tukey post hoc test. Normality and homoscedasticity requirements were met by applying an arcsin transformation to the data. Statistical analyses were performed using SPSS (v.15.0) software.

2. RESULTS

2.1. Multielement Analysis and Ash Contents

Table S1. Multielement analysis of *D. raddiana* plant components and ashes. Contents are expressed in ppm.

Element	Plant			Ashes		
	Leaves	***Stems***	***Roots***	***Leaves***	***Stems***	***Roots***
Bal	888777	952820	951772	726030	810775	771435
Mg	1488	1531	1791	7978	14452	7069
Al	593	846	2216	936	1021	11286
Si	77424	25056	18090	205574	94347	136087
P	1481	935	1207	4517	6853	6282
S	4105	1552	2832	4593	7520	4995
Cl	3219	835	636	170	495	241
K	17238	13352	5439	24367	38095	19785
Ca	8501	9019	12534	25049	25751	17181
Sc	<LOD	<LOD	<LOD	77	<LOD	<LOD
Ti	627	110	522	247	387	4293
Cr	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Mn	<LOD	<LOD	148	<LOD	<LOD	673
Fe	213	85	2698	425	360	22307
Cu	<LOD	20	<LOD	22	33	54
Zn	75	48	55	118	123	118
As	<LOD	<LOD	<LOD	30	<LOD	<LOD
Rb	6	8	14	12	19	12
Sr	11	9	19	24	23	30
Zr	8	8	9	4	4	30
Mo	5	4	<LOD	<LOD	<LOD	<LOD
Pb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Bal (balance), i.e., the difference to 100% (1,000,000 ppm) of the sum of all measured elements, includes elements with atomic number $Z \leq 11$ and mainly accounts for organic matter (H, C, N, O). '<LOD' indicates contents below the limit of detection.

2.2. Vibrational Spectra

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra of all of the plant parts (Figure S2) showed in common bands at 3254–3273, 2927–2929, 1586–1590 (shifted to 1583 cm^{-1} in ashes), 1371–1394, 1259–1261, 1024–1028, 918–923, 862–869, 816–817, and 769–776 cm^{-1}.

The broad and intense band near 3260 cm^{-1} should be attributed to OH stretching and that at 2928 cm^{-1} to the methylene (CH_2) stretching features. The band at 1590 cm^{-1} suggests the presence of COO^- and can be assigned to the stretching of the carboxyl group. The band at 1394 cm^{-1} should be ascribed to OH in-plane stretching. The band at 1260 cm^{-1} may be attributed to C–C–O asymmetric stretching from acetylated glucomannan (which is a hemicellulose component), and the one at 1026 cm^{-1} to the C–O stretching of secondary alcohols (Maréchal and Chanzy, 2000). Moreover, the spectrum of large leaves exhibited a distinct shoulder at 1100 cm^{-1} that can be assigned to glycerine when this phytochemical appears in a mixture in a very significant percentage (Pérez et al., 2009).

In the lower wavenumber region, the band at 918 cm^{-1} arises from bending vibrations of the C–H group, and the ones at 817 and 868 cm^{-1} are characteristic of the vibration anomeric region of carbohydrates or C–H deformation. The CH_2 vibration in cellulose I α is sensitized by the band at 770 cm^{-1}.

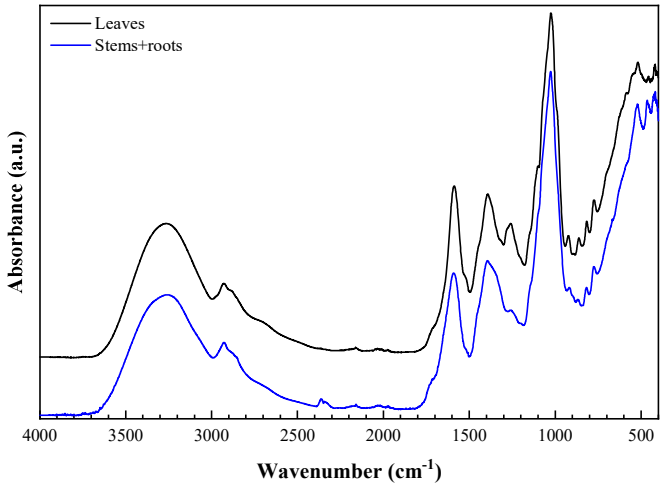

Figure S2. ATR-FTIR spectra of *D. raddiana* plant parts.

2.3. Thermal Behaviour

Thermogravimetric (TG) curves of *D. raddiana* leaves (Figure S3) showed a first loss associated with absorbed moisture, 2.9% at 149 °C; a second loss (at 453 °C) of 51.2%, sensitized by a derivative thermogravimetric (DTG) peak at around 300 °C (autoignition of glycerine); and a third step that corresponded to the end of the decomposition (maximum at 490 °C), and which led to a weight loss of 8.7%.

Although the TG curve of *D. raddiana* stems and roots was similar to those discussed above in the first step (weight loss of 3.4%), it significantly differed in the second step, with a more delayed beginning of the decomposition (at 172 °C), a lower mass change (47.4%) and a splitting of the DTG maximum into two peaks, at 290 and 330 °C. The weight loss at 456 °C was 21.9%.

These thermal effects evidenced that, under heating, both changes in composition and darkening occur on *D. raddiana* plant parts due to the modification of the hemicelluloses structure, migration of extractives to the surface, boiling of components, and formation of oxidative products (such as acrolein from glycerine at 280 °C).

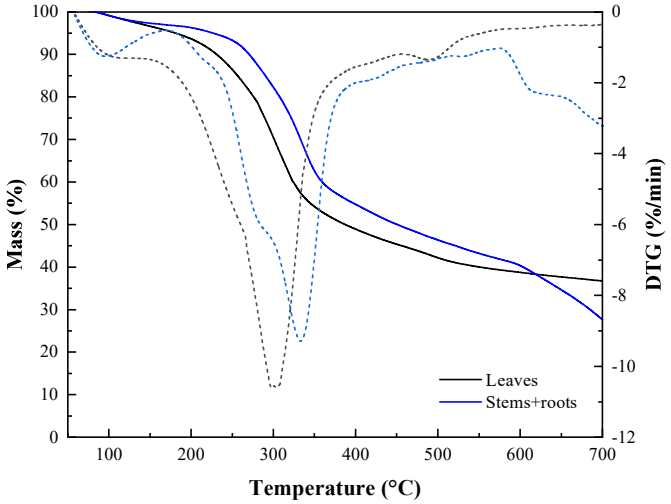

Figure S3. Thermal behaviour (TG and DTG curves) of *D. raddiana* leaves and stems and roots.

2.4. Extract Characterization by GC–MS

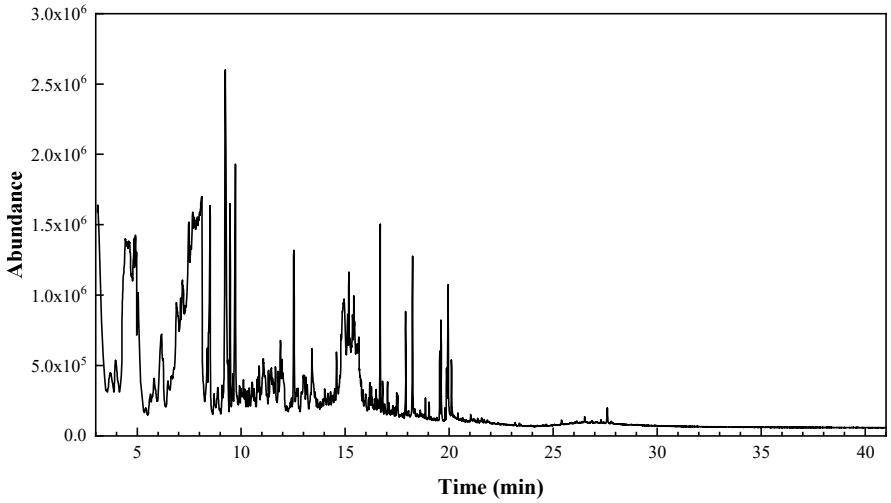

Figure S4. GC–MS chromatogram of *D. raddiana* leaves extract.

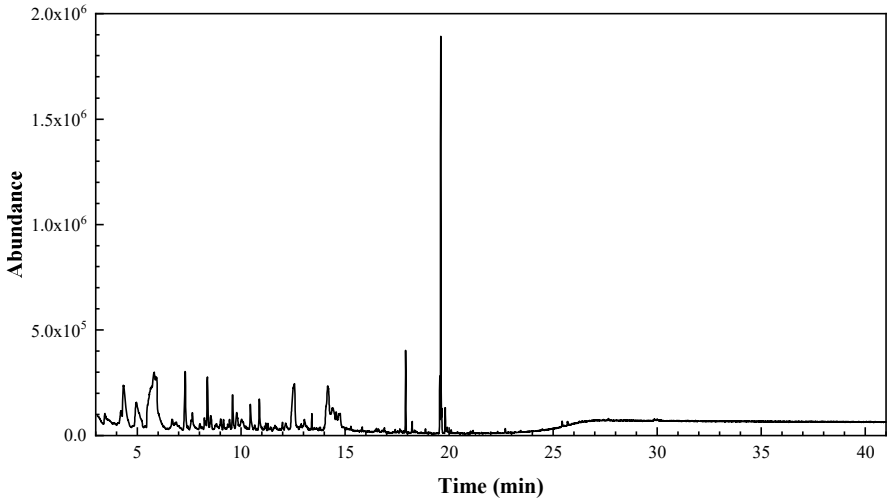

Figure S5. GC–MS chromatogram of *D. raddiana* stems and roots extract.

Table S2. Chemical species identified in *D. raddiana* leaves extract.

RT (min)	Area (%)	Assignment
3.7220	0.4899	Furfural
3.9653	0.7319	2-Furanmethanol
4.4342	5.9999	Propane, 1,3-dimethoxy-
4.6242	3.9484	Hydrazine, ethyl-
4.8616	2.0082	E-1-Methoxy-3-pentene
4.9209	1.3975	Butane, 1,4-bis(5-thioxo-1,2,4-triazol-2-in-3-yl)-
4.9684	0.7231	2-Butenoic acid, 3-methyl-
5.0396	2.1293	[1,3,4]Thiadiazol, 2-amino-5-(2-piperidin-1-ylethyl)-
5.4135	0.0559	α-Aminooxy-propionic acid, ethyl ester
5.6450	0.2576	Imidazole, 1,4,5-trimethyl-
5.8171	0.9107	Phenol
6.1733	1.7870	β-Alanine, N-ethyl-, ethyl ester
6.2504	0.4640	Butanoic acid, 2-oxo-
6.4878	0.6595	Xylitol
8.0073	28.2385	Glycerine
8.3753	0.7240	2-Propanamine, N-methyl-N-nitroso-
8.5118	2.1043	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
8.7076	0.2269	Benzoic acid
8.8857	0.4151	2(3H)-Furanone, dihydro-4-hydroxy-
9.0934	0.2305	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
9.2418	4.0937	Catechol
9.3605	0.3336	Diglycerol
9.4674	1.0464	Benzofuran, 2,3-dihydro-
9.6098	0.3765	l-Alanine, N-allyloxycarbonyl-, pentyl ester
9.7285	2.2205	5-Hydroxymethylfurfural
9.9303	0.2675	Benzeneacetic acid
9.9956	0.2809	Undecanal
10.1203	0.3845	1,2-Benzenediol, 3-methyl-
10.1915	0.1883	Naphthalene, 2,6-bis(1,1-dimethylethyl)-
10.2983	0.2324	D-Glucitol, 4-O-hexyl-
10.3933	0.2699	Benzenethiol, o-isopropyl-,
10.5357	0.3974	1,2-Benzenediol, 4-methyl-
10.5891	0.1120	Lauryl acetate
10.6366	0.1487	Hydroquinone
10.6782	0.1273	2-Nonen-4-yn-1-ol, (Z)-
10.7731	0.2443	5-Acetoxymethyl-2-furaldehyde
10.8265	0.2065	N-Cyanomethyl-N-methylacetamide
10.8740	0.3917	(S)-(-)-1,2,4-Butanetriol, 2-acetate
10.9512	0.1793	d-Glycero-d-galacto-heptose
11.0699	0.9619	d-Mannitol, 1,4-anhydro-
11.1530	0.3821	6-Desoxy-l-gulitol
11.3192	0.2304	2-Amino-2-thiazoline-4-carboxylic acid
11.4616	0.8009	2-Propyl-1-pentanol, methyl ether
11.5151	0.3510	1-Deoxy-d-arabitol
11.6041	0.1859	α-D-Mannopyranoside, methyl 3,6-anhydro-
11.6575	0.6069	Cyclopentene, 3-propyl-
11.7881	0.3672	1-(2,4-Dimethyl-furan-3-yl)-ethanone
11.8949	0.6844	Malonic acid, 1,3-dithio-, bimol. cyclic S,S-ethylene ester
11.9602	0.3867	1,2,3-Benzenetriol
12.0136	0.6171	3-Nonene, (E)-
12.1857	0.0693	D-Glucose, 2,3,4,5,6-pentaacetate
12.2095	0.0435	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-
12.3697	0.1794	Acetic acid, decyl ester
12.4232	0.1403	Furan, 2-methoxy-5-methyl-4-phenyl-
12.5359	1.0805	2,1,3-Benzothiadiazole
12.6487	0.2017	Cyclohexane, 1R-acetamido-4cis-acetoxy-5,6Zcisepoxy-2cis,3trans-dimethoxy-
12.6962	0.1427	Methylparaben
12.9098	0.2356	Dichloroacetic acid, 2-pentadecyl ester
12.9989	0.4139	Dodecanoic acid, 4-methyl-, methyl ester
13.0404	0.3415	Octanoic acid, 4-methyl-, methyl ester

13.1235	0.2141	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one
13.1769	0.3425	Sulphurous acid, nonyl 2-propyl ester
13.4025	1.2015	Phenol, 2,4-bis(1,1-dimethylethyl)-
13.5568	0.2549	2-Amino-5-methyl-4-oxo-3,4-dihydropyrimidine
13.6695	0.1529	8-Oxabicyclo[5.1.0]oct-5-en-2-ol, 1,4,4-trimethyl-
13.7526	0.1569	Octan-2-one, 3,6-dimethyl-
13.8120	0.1344	5-Ethyl-3-hepten-2-one
13.9307	0.2068	Cyclohexanol, 1R-4-acetamido-2,3-cis-epoxy-
13.9841	0.0985	exo-2-Methyl-endo-2-(acetylamino)norbornane
14.1681	0.2864	Formamide, N,N'-2,6-piperazinediylidenebis-
14.2394	0.1051	Hexanoic acid, 5-oxo-, ethyl ester
14.3046	0.2316	6-(5-Methyl-furan-2-yl)-hexan-2-one
14.3640	0.1092	α-Santoline alcohol
14.4174	0.2059	1-Methoxy-1,4-cyclohexadiene
14.5064	0.3212	Cyclopentanecarboxylic acid, 3-methyl-4-methylene-, methyl ester
14.5895	0.6571	1-(2,4-Dimethoxyphenyl)-propan-2-one
14.9160	2.3977	Methyl isopropylidene-β-d-arabinoside
14.9457	1.6742	Butyraldehyde, semicarbazone
15.1237	1.0309	Benzene, 1,2-diethyl-3,4-dimethyl-
15.1831	0.7997	2-Isobutyl-3-methylpyrazine
15.2246	0.6950	1,5-Anhydro-d-mannitol
15.3552	1.1332	N-Methoxymethyl-N-methylacetamide
15.4205	1.5577	Cyclopropane, 1,1-dimethyl-2-(1-methylethoxy)-3-(3-methyl-1-pentynyl)-
15.5273	0.3853	2,3-Epoxybutane
15.5807	0.5874	2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl]guanine
15.6579	1.1484	3-Deoxy-d-mannoic lactone
15.7766	0.3319	3,5-Dihydroxyphenylacetic acid, methyl ester
16.1327	0.1697	2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester
16.1921	0.2719	trans-Z-α-Bisabolene epoxide
16.2633	0.2930	2-Butenoic acid, 3-methyl-, methyl ester
16.4176	0.1568	7,7-Dimethyl-4-(2-methyl-4-thiazolyl)-1,8-dioxaspiro[4.5]decan-2-one
16.4888	0.2033	Spiro[2.5]octane, 5,5-dimethyl-4-(3-oxobutyl)-
16.5541	0.1079	2-Cyclopenten-1-one, 4-butyl-3-methoxy-
16.6075	0.1207	4-(1,5-Dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one
16.6847	0.8366	Phenol, 3-isopropoxy-5-methyl-
16.8034	0.3042	2-Butenoic acid, 1-methylethyl ester
16.9043	0.2057	5,5,8a-Trimethyldecalin-1-one
17.0408	0.1633	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1α,2β,5α)-
17.1061	0.1525	Tartaric acid, dimenthyl ester
17.2248	0.1052	1-Hydroxy-p-menth-3-one
17.2960	0.1344	2(1H)-Naphthalenone, octahydro-8a-methyl-, trans-
17.4088	0.0745	Phthalic acid, butyl tetradecyl ester
17.4860	0.1323	6-Nonen-1-ol, (E)-
17.5275	0.1442	Propenone, 3-(2-benzoxazolylthio)-1-phenyl-
17.6700	0.0625	sesquicineole
17.7174	0.0619	Z-12-Tetradecenal
17.8065	0.0553	Methyl undecyl ether
17.9133	0.4239	Hexadecanoic acid, methyl ester
17.9845	0.0714	3,7-Benzofurandiol, 2,3-dihydro-2,2-dimethyl-
18.0498	0.1099	Cyclohexaneethanol, 4-methyl-β-methylene-, trans-
18.2457	0.8445	n-Hexadecanoic acid
18.3466	0.1246	Benzenemethanol, 4-(1,1-dimethylethyl)-
18.5662	0.0400	Cyclotetradecane
18.6137	0.0896	2H-1-Benzopyran-2-one, 7-hydroxy-6-methoxy-4-methyl-
18.6849	0.0799	Cyclopropanol, 1-(3,7-dimethyl-1-octenyl)-
18.7917	0.0672	3-(2-Methoxymethoxyethylidene)-2,2-dimethylbicyclo[2.2.1]heptane
18.8630	0.1162	Isopropyl palmitate
19.0351	0.1255	p-Amidinobenzamide
19.1300	0.0474	Neoclovene oxide
19.2725	0.0286	7-Thiabicyclo[4.2.1]nonane
19.4090	0.0429	7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin
19.5455	0.2473	Methyl 10-trans,12-cis-octadecadienoate
19.6108	0.5825	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-

19.8126	0.0599	Methyl stearate
19.8838	0.2479	9,12-Octadecadienoic acid (Z,Z)-
19.9491	0.9068	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
20.1153	0.3187	Octadecanoic acid
20.6554	0.0371	1,4-Methanoazulen-7-ol, decahydro-4,8,8,9-tetramethyl-, (+)-
20.8275	0.0361	Oxirane-2-carboxylic acid, 2-aminocarbonyl-3-methyl-3-(1-methylethyl)-, ethyl ester
21.0353	0.0819	5-Methyl-isoxazolidin-3-one
21.1718	0.0375	1-(3,3-Dimethyl-1-yl)-2,2-dimethylcyclopropene-3-carboxylic acid
21.3736	0.0404	(7R,8S)-cis-anti-cis-7,8-Epoxytricyclo[7.3.0.0(2,6)]dodecane
21.4982	0.0592	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene
21.5635	0.0522	Eicosanoic acid, methyl ester
21.6882	0.0462	3α,4α-Epoxymurolan-9(11)-en-10-ol
21.8365	0.0255	6-Nonenal, 3,7-dimethyl-
22.3351	0.0062	trans-2,3-Methylenedioxy-b-methyl-b-nitrostyrene
23.1720	0.0163	Docosanoic acid, methyl ester
23.3738	0.0084	Adamantane, 1-isothiocyanato-3-methyl-
25.4036	0.0217	1H-Indole, 5-methyl-2-phenyl-
26.1396	0.0182	2-Ethylacridine
26.8637	0.0146	1H-Indole, 1-methyl-2-phenyl-

Table S3. Chemical species identified in *D. raddiana* stems and roots extract.

RT (min)	Area (%)	Assignment
3.4489	1.2526	Ethane, 1,1,1-triethoxy-
4.2205	1.3904	N-Hydroxy-N-ethylcarbamic acid, 2-(methoxycarbonylamino)ethyl ester
4.3451	6.2954	Dihydroxyacetone
4.9624	5.3867	2-Cyclopenten-1-one, 2-hydroxy-
5.7043	1.9673	Diglycerol
5.8230	20.0201	Glycerine
6.6836	0.7469	Piperazine, 1,4-dimethyl-
6.8320	0.4818	2-Pyrrolidinone, 1-methyl-
6.8854	0.2473	N-Acetyl-d-galactosamine
7.3128	3.5861	1,3,5-Triazine-2,4,6-triamine
7.4374	0.2213	1,3-Dioxane, 2,4-dimethyl-
7.6036	0.5046	3-Heptanol, 2,4-dimethyl-
7.6689	1.2611	Undecane
8.0250	0.4605	c-(3-Methyl-4,5-dihydroisoxazol-5-yl)methylamine
8.2387	0.5258	2-Propanamine, N-methyl-N-nitroso-
8.3811	2.6251	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
8.5473	1.0886	2(3H)-Furanone, dihydro-4-hydroxy-
8.7610	0.2970	N-Acetylmannosamine
8.8144	0.5740	Pyrrolidin-1-acetic acid
9.0221	0.8441	8-Methyl-hexahydro-pyrano[3,2-b]pyran-2-one
9.1586	0.5446	Catechol
9.3605	0.3203	Thiophene, tetrahydro-, 1,1-dioxide
9.4436	0.5769	Benzofuran, 2,3-dihydro-
9.5919	1.7876	5-Hydroxymethylfurfural
9.7937	1.8243	1,2,3-Propanetriol, 1-acetate
10.0311	1.7250	Erythritol
10.4526	1.5514	Glucuronamide
10.6603	0.3127	1,3-Dioxolane, 4-methyl-
10.8799	1.6316	α-d-Ribopyranoside, methyl
11.1351	6.8616	D-erythro-Pentose, 2-deoxy-
11.2953	0.2126	2-Hydroxyisocaproic acid, methyl ether, methyl ester
11.4200	0.1867	Ethyl 3-cyclohexenecarboxylate
11.5565	0.1381	Octahydropyrrolo[1,2-a]pyrazine
11.6277	0.1843	α-d-Lyxofuranoside, methyl
11.6871	0.1060	Cyclopentane-1,2-diol
11.9898	0.3326	Benzene, 1-chloro-4-methoxy-
12.1441	0.5670	Propanamide, N,N-dimethyl-
12.6724	0.3095	Eugenol
12.8148	0.1113	β-d-Lyxofuranoside, methyl, triacetate
12.8920	0.3544	Cyclodecane

13.0463	0.9505	3,4-Altrosan
13.4024	0.3782	Phenol, 2,4-bis(1,1-dimethylethyl)-
13.7645	0.0708	Acetyl turicine
13.9781	0.0816	Benzoic acid, 4-hydroxy-3-methoxy-
14.1740	6.5289	β-D-Glucopyranoside, methyl
14.3105	0.5167	N-Acetyl-d-serine
14.3877	1.4562	2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl]guanine
14.4292	1.6539	Galactitol
14.5717	1.2213	Octanoic acid
14.7022	1.7881	α-D-Xylofuranoside, methyl
15.2839	0.1589	Ethanone, 1-(2,5-dimethoxyphenyl)-
15.8240	0.1269	Tridecanoic acid, 12-methyl-, methyl ester
16.8924	0.1597	Methyl 9-methyltetradecanoate
17.4087	0.1139	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester
17.6343	0.0964	Methanimidamide, N'-(3-hydroxyphenyl)-N,N-dimethyl-
17.9132	1.8739	Hexadecanoic acid, methyl ester
18.2219	0.2732	n-Hexadecanoic acid
18.8629	0.1191	i-Propyl 14-methyl-pentadecanoate
19.5514	1.1719	9,12-Octadecadienoic acid, methyl ester, (E,E)-
19.5988	9.3380	cis-13-Octadecenoic acid, methyl ester
19.6463	0.6164	5-Undecyne
19.8125	0.5511	Methyl stearate
19.9016	0.2488	Oleic Acid
20.0974	0.0732	Pentadecanoic acid
22.7030	0.1156	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
25.4333	0.2474	Benzo[h]quinoline, 2,4-dimethyl-

Table S4. Effect of the plant lyophilizate and extract on sperm motility and viability. Results are expressed as mean ± SEM (n = 3).

Incubation Time (h)	Sample	Total Motility (%)	Progressive Motility (%)	Rapid Spermatozoa (%)	Viability (%)
0	L	85.60 ± 0.49	38.41 ± 0.35	72.64 ± 0.42	83.13 ± 0.26
	C_L	89.85 ± 0.15	35.55 ± 0.14	77.05 ± 0.21	84.96 ± 0.23
	E	86.49 ± 0.44	53.12 ± 0.30	62.03 ± 0.57	83.30 ± 0.27
	C_E	80.70 ± 0.69	36.50 ± 0.29	68.84 ± 0.67	82.04 ± 0.22
1	L	80.61 ± 0.45	30.72 ± 0.60	66.90 ± 0.86	79.15 ± 0.42
	C_L	84.58 ± 0.14	33.06 ± 0.49	71.23 ± 0.43	82.65 ± 0.29
	E	88.00 ± 0.25	51.96 ± 0.24	52.41 ± 0.57	83.65 ± 0.30
	C_E	77.55 ± 0.50	35.04 ± 0.25	65.18 ± 0.62	83.03 ± 0.30
2	L	79.30 ± 0.58	25.93 ± 0.51	68.34 ± 0.70 a	81.65 ± 0.30
	C_L	86.08 ± 0.14	36.97 ± 0.93	75.42 ± 0.31 a	81.05 ± 0.38
	E	76.14 ± 0.52	44.94 ± 0.54	29.39 ± 0.77 b	84.57 ± 0.33
	C_E	80.86 ± 0.54	34.75 ± 0.60	66.52 ± 0.74 a	83.76 ± 0.29
3	L	80.06 ± 0.13	36.74 ± 0.73	66.35 ± 0.24 a	78.98 ± 0.26
	C_L	74.89 ± 0.87	27.80 ± 0.30	63.99 ± 0.83 a	81.72 ± 0.40
	E	70.36 ± 0.76	39.37 ± 0.82	22.33 ± 0.90 b	83.90 ± 0.38
	C_E	75.89 ± 0.79	39.18 ± 0.51	64.82 ± 0.79 a	88.91 ± 0.40
4	L	78.23 ± 0.44	29.41 ± 0.49	63.73 ± 0.58 a	75.66 ± 0.35
	C_L	78.28 ± 0.26	25.03 ± 0.48	67.47 ± 0.44 a	78.73 ± 0.45
	E	71.06 ± 0.57	30.54 ± 0.63	10.55 ± 0.27 b	83.91 ± 0.33
	C_E	73.10 ± 0.77	31.11 ± 0.55	57.57 ± 1.03 a	82.34 ± 0.33

L: 100 µL of lyophilizate (6 mg·mL^{-1} in PBS); C_L (control for L): 100 µL of PBS added; E: 100 µL of extract (6 mg·mL^{-1}), and C_E (control for E): 100 µL of bi-distilled water. Means followed by a common letter are not significantly different by Tukey's test at the 5% level of significance.