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ORIGINAL ARTICLE

Optimization of a novel *Renealmia ligulata* (Zingiberaceae) essential oil extraction method through microwave-assisted hydrodistillation

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Abstract

Renealmia is a tropical plant genus within the Zingiberaceae family. In tropical South America, *Renealmia* plants are known for their therapeutic uses against bone and muscle pain, colds, and to counteract snake bite symptoms. Despite the biomedical importance of *Renealmia* metabolites, the components of their essential oils (EO) have been scarcely studied, and a cause thereof is the lack of local efficient, inexpensive, and environmentally friendly EO extraction methods. This work addressed the optimization of an EO extraction method from the aerial parts and rhizomes of *Renealmia ligulata* plants based on microwave-assisted hydrodistillation (MAHD) with an ultrasound-assisted extraction (UAE) pretreatment. Three MAHD extraction variables (radiation power, radiation exposure length, and solvent volume) were studied on their own and in combination using a response surface analysis to determine the value combinations leading to optimal EO yields. The results showed that the best average extraction duration time was 42.5 min, combined with a radiation power of 765 W and a solvent volume 225.9 mL for 30 g of aerial part plant material or 799 W and 145 mL of solvent for 20 g of plant rhizomes. A GC-MS analysis of the obtained *R. ligulata* EOs revealed that their main component was epi-Eudesmol (28% in plant aerial parts and 13% in rhizomes), which is a molecule of interest considering its reported neuro-protective properties.

Keywords: epi-Eudesmol; essentials oils; microwave-assisted hydrodistillation; *Renealmia*, response surface analysis; ultrasound-assisted. .

1. Introduction

The Zingiberaceae family, distributed throughout regions of Africa and South America, comprises nearly 85 species and entails 23 genera native species of Africa and 62 of the American tropics [1]. Within this family, several species of the *Renealmia* genus, native to the Amazon, are frequently employed as medicines by indigenous peoples. For instance, the Kichwa in Ecuador, use these plants to treat colds, as analgesics for bone and muscle pain, and to counteract snake bite symptoms [2].

The species *Renealmia ligulata* (Maas) occurs in the Colombian Andes at altitudes between 1,200 and 2,000 meters above sea level, bearing the vernacular names of Murrapa or Matandrea. In the Colombian department of Quindío, in the Andean middle range, this plant species has been recorded in the municipalities of Armenia, Buenavista, Calarcá, Circasia, Córdoba, Filandia,

Génova, Pijao, and Salento, at altitudes between 1,450 and 2,050 m.a.s.l., and it is frequently associated with roadsides, water bodies, and inside forests at the edge of trails on slopes, forming conspicuous aggregates.

The essential oils (EOs) of *Renealmia* species have been scarcely investigated, yet the most important work conducted in the *Renealmia alpinia* (Rottb.) Maas species, revealed that its rhizomes chiefly contain caryophyllene (22.9%), β-pinene (12.0%), espatulenol (10.0%), aromandrene (8.3%), and γ-cadinene (5.3%) [3].

EOs are a complex mixture of secondary metabolites, entailing monoterpenes, sesquiterpenes, phenylpropanoids, and some compounds of non-terpenoid nature. Given this ample EO array in medicinal and aromatic plants, ethnic and traditional wisdom has stirred the study of their biological properties as well as their antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [4].

Extraction is one of the fundamental steps in plant EO research and use. The efficiency of different conventional EO extraction techniques, like extraction with solvents, distillation, and conventional hydrodistillation (HD), is usually affected by extraction-inherent features. For instance, lengthy sample exposure to solvents or thermal treatment in HD yields, likely results in plant metabolite degradation [5, 6].

Novel approaches have been applied in EO extraction from plants, such as microwave-assisted hydrodistillation (MAHD), which is a relatively inexpensive and environmentally friendly method. In addition, MAHD provides several advantages for EO extraction because it can reduce heating times, lowers energy consumption, and overcomes the inconveniences already mentioned [7, 8, 9, 10].

Likewise, seeking to improve yields and to shorten extraction times, various researchers have applied ultrasound-assisted extraction (UAE) to EO. This is a modern extraction technology that uses the cavitation, mechanical vibration, and thermal effects produced by ultrasound to destroy plant cell walls, thus enhancing solvent diffusion and accelerating the dissolution of target compounds. This method has the advantages of simple instrumentation, easy operation, and high efficiency [11, 12, 13, 14].

An important part in EO extraction though MAHD is the optimization of extraction variables. These include extraction time, microwave power, and the plant material-solvent ratio. The response surface methodology (RSM) is a valuable statistical and mathematical tool widely used to predict the optimal experimental parameters and construct a mathematical model to analyze the effects of interactions among variables [14].

This work focused on (i) optimizing the extraction process of EO from *Renealmia ligulata* aerial parts and rhizomes, using RSM in the MAHD context, (ii) assessing the effect of UAE as an EO extraction pretreatment about yield percentages when applying the MAHD extraction technique, and (iii) studying the chemical composition, through gas chromatography mass spectrometry (GC-MS), of the extracted EOs.

2. Material and Methods

2.1. Plant material collection and identification

Plant material, consisting of plant aerial parts (*i.e.*, flowers, leaves, and stems) and rhizomes, were collected in the pristine forest of La Concha (4° 38' 12" N y 75° 39' 06" W), within the Circasia municipality (western slope of the central mountain range of Colombia) in the Colombian department of Quindio. For taxonomic identification, a complete specimen was taken to the herbarium of the University of Quindio (HUQ) to be analyzed by a botanical expert in this plant group. The material was entered to the herbarium collection with accession number 029953, thus confirming its taxonomic identity as *R. ligulata* Mass material (a taxonomic description of the plant species in question is provided in supplementary information).

2.2. Material drying and grinding

R. ligulata aerial parts and rhizomes were subjected to drying in a recirculating air oven at temperatures between 40 and 45 °C for eight days; thereafter, the plant material was pulverized by using a fodder grinding mill TRF 70.

2.3. Microwave-assisted Hydrodistillation (MAHD) extraction

A household microwave oven (General Electric, JES11G, with a maximum power of 1,000 W; capable of 100-W increments and with a frequency of 2450 MHz) was modified for the procedure. The microwave oven was adapted for extraction by drilling an opening and installing a Clevenger-type condenser on top. Then, a flat-bottom flask containing the pulverized plant material was placed inside the appliance and the MAHD was carried out [15]. The EOs obtained were stored at 4 °C until analysis. The EO yield was calculated following Equation 1.

$$\%R = \frac{(mass(g)of\,EO)}{(mass(g)plantmaterial)} * 100 \quad (1)$$

The adapted MAHD system was specific for the chosen microwave oven brand. The modifications made to the appliance may not apply when adapting another of a different brand. Thus appliance technical specification must be taken into account when constructing a similar experimental setup. This is due to the lack of empirical evidence supporting a linear correlation between their power and efficiency concerning energy conversion rate.

2.4. MAHD extraction optimization via RSM with a Box–Behnken experimental design

A Box-Behnken design (BBD) was implemented with three factors: length of microwave radiation, microwave power, and amount of solvent, on three levels each [low (-1), medium (0), and high (+1)] to investigate simultaneously the effects of the independent factors on EO yields in the context of MAHD extraction. These factors were coded, as shown in **Table 1**. The variables were taken from different works conducted with some modifications [14, 16, 17, 18].

Table 1. Evaluated EO extraction variables and their levels depending on plant part employed

Extraction method independent variable	Plant part					
	Aerial parts (30 g)			Rhizomes (20 g)		
Levels	-1	0	1	-1	0	1
Microwave radiation length (A) (min)	25	35	45	25	35	45
Microwave power (B) (W)	600	700	800	600	700	800
Solvent volume (C) (mL)	150	225	300	100	150	200

This design evaluated the EO percentage of yield as the response variable. The study predicted 12 runs, together with three random replicates at the central point (CP) applied to test the experimental error.

2.5. MAHD with ultrasound pretreatment (MAHD+US)

Ultrasound pretreatment was applied on *R. ligulata* aerial parts (30 g) and rhizomes (20 g). Each plant material type was subjected to an ultrasound bath for 30 minutes in a Bransonic equipment series M1800-H, set to a frequency of 40-KHz and 145-W power [19].

2.6. GC-MS Analysis

The obtained EO were analyzed via GC-EM in a SHIDMAZU-QP2010 apparatus employing helium as carrier gas with a flow of 1 mL/min. All samples were prepared by diluting in 1:20 v/v ratio with dichloromethane and 1 μl of each sample was injected in Split mode. A Zebron ZB-5MS 5 % Polysiralene – 95 % Polydimethylsiloxane column was used (30 m $\times$ 0.25 mm ID $\times$ 0.25 μm). The temperatures of the column, injector, and transfer line were adjusted to 40, 300, and 300 °C, respectively. The heating ramp was programmed in the following manner: initially, the column temperature was set at 40 °C constant for 2 min, then increased to 260°C with 5°C/min steps; and finally rose up to 310 °C with increasing steps of 20 °C/min; remaining unaltered for 3 min. Mass spectrometry was conducted with an electron impact ionization system at 70 eV, in Scan mode, with an analysis range from 40 m/z to 450 m/z. The EO components were identified comparing the obtained retention times and mass spectra to the equipment's 2013 NIST library and through comparison of Kovats indices, which were determined based on the retention times of n-alkanes C_8 - C_{25}, according to Adams [20].

3. Results and Discussion

3.1. EO yields from MAHD-assisted extraction

The Box-Behnken design to assess three variables affecting EO extraction efficiency (*i.e.*, percentage of EO yield) with the MAHD method, resulted in the data shown in **Table 2** and **Table 3**, which reveal *R. ligulata* aerial parts and rhizomes, respectively, as EO sources.

The EOs from *R. ligulata* revealed a better yield from plant aerial parts compared to rhizomes. The results obtained in the experimental design were subjected to analysis of variance (ANOVA) in the Stat graphics program, as shown in **Table 4** and **Table 5**.

Table 2. Result of Box – Behnken experimental design for EO extraction yield from *R. ligulata* aerial parts.

Radiation power (W)	Radiation length (min)	Solvent volume (mL)	% Yield (AP)
700 (CP)	35	225	0.1708
700	25	150	0.1216
600	35	300	0.1576
700	25	300	0.1386
800	25	225	0.1483
700 (CP)	35	225	0.1712
600	45	225	0.1456
700	45	300	0.1726
700 (CP)	35	225	0.1668
800	35	300	0.1706
800	45	225	0.1796
600	25	225	0.1276
700	45	150	0.1681
600	35	150	0.1465
800	35	150	0.1682

Table 3. Result of Box – Behnken experimental design for EO extraction yield from *R. ligulata* rhizomes.

Radiation power (W)	Radiation length (min)	Solvent volume (mL)	% Yield (Ri)
700 (CP)	35	150	0.0526
600	25	150	0.0303
700	25	200	0.0332
700	25	100	0.0391
800	25	150	0.041
700	45	100	0.0503
700	45	200	0.0511
800	35	100	0.0582
800	35	200	0.0541
600	45	150	0.0452
700 (CP)	35	150	0.052
600	35	100	0.0412
700 (CP)	35	150	0.0515
600	35	200	0.0428
800	45	150	0.0611

Table 4. ANOVA for EO extraction yields from *R. lingulata* aerial parts

Source	Sum of squares	Mean square	F-ratio	p value
A: Radiation power	9.99E-04	9.99E-04	168.76	0.0059
B: Radiation length	2.11E-03	2.11E-03	355.74	0.0028
C: Solvent volume	1.53E-04	1.53E-04	25.87	0.0366
AA	7.19E-05	7.19E-05	12.14	0.0734
AB	4.42E-05	4.42E-05	7.47	0.1119
AC	1.89E-05	1.89E-05	3.20	0.2157
BB	8.21E-04	8.21E-04	138.70	0.0071
BC	3.91E-05	3.91E-05	6.60	0.1240
CC	7.35E-05	7.35E-05	12.42	0.0719
Lack of fit	1.80E-04	5.99E-05	10.12	0.0913
Pure error	1.18E-05	5.92E-06		
Total (corr.)	4.45E-03			
R^2				95.69%
R^2 adjusted				87.94%

Table 5. ANOVA for EO extraction yields from *R. lingulata* rhizomes

Source	Sum of squares	Mean square	F-ratio	p value
A: Radiation power	3.77E-04	3.77E-04	1242.04	0.0008
B: Radiation length	5.14E-04	5.14E-04	1693.19	0.0006
C: Solvent volume	7.22E-06	7.22E-06	23.8	0.0395
AA	3.63E-06	3.63E-06	11.97	0.0743
AB	6.76E-06	6.76E-06	22.29	0.0421
AC	8.12E-06	8.12E-06	26.78	0.0354
BB	1.63E-04	1.63E-04	536.95	0.0019
BC	1.12E-05	1.12E-05	37	0.026
CC	1.43E-05	1.43E-05	47.08	0.0206
Lack of fit	5.56E-06	1.85E-06	6.11	0.1439
Pure error	6.07E-07	3.03E-07		
Total (corr.)	1.10E-03			
R^2				99.44%
R^2 adjusted				98.43%

Tables 4 and 5 show the effect of the sources of variance on EO yield, when extracted from *R. lingulata* aerial parts and rhizomes; testing the statistical significance of each extraction variable effect by comparing its mean square against an estimate of the experimental error. In addition, the R^2 statistic indicates that the model adjusted explains 95.6 % and 99.4 % of the variability in EO yield from plant aerial parts and rhizomes, respectively. Likewise, the p value for the lack of fit in the ANOVA table is > 0.05, suggesting that the model is adequate for the data observed within the 95% confidence interval (CI).

As shown in **Fig.1**, four effects were significant ($p < 0.05$) on EO exaction yields from plant aerial parts, whereas eight effects were significant on EO exaction yields from rhizomes. These effects were significantly different from zero, within the 95 % CI, as shown in the Pareto diagram in Figs 1A and 1B.

3.2. MAHD extraction variable effects as revealed by surface response graphics

To assess the interactions between the extraction parameters and the response variable (*i.e.*, EO extraction yield), multiple response surface (RS) graphics were constructed and analyzed **Fig. 2**. Figures 2A and 2B show the effect of microwave radiation, in terms of radiation power and the length of radiation exposure, on EO yield, from plant aerial parts and rhizomes, respectively. A microwave exposure time above 35 with a radiation power between 720 and 800 W resulted in the best best yields. These outcomes may be due to the microwave effect, promoting rotation of the molecular dipoles and, thus, internal heating within a short time, resulting in a high-pressure gradient within the plant material, releasing EO [19].

Regarding the combination of the length of radiation exposure and solvent amount, the RS graph ics (Figs. 2C and 2D) revealed that an exposure time above 35 min with solvent volumes between 180 and 240 mL for the aerial parts and between 120 and 140 mL for rhizomes resulted in best EO yields. The highest solvent volumes slowed down the process and led to suboptimal EO yields. Hence, at a longer extraction time with the least and upper solvent amounts can shorter extraction time but may lead to incomplete release and possibly degradation of metabolites [21].

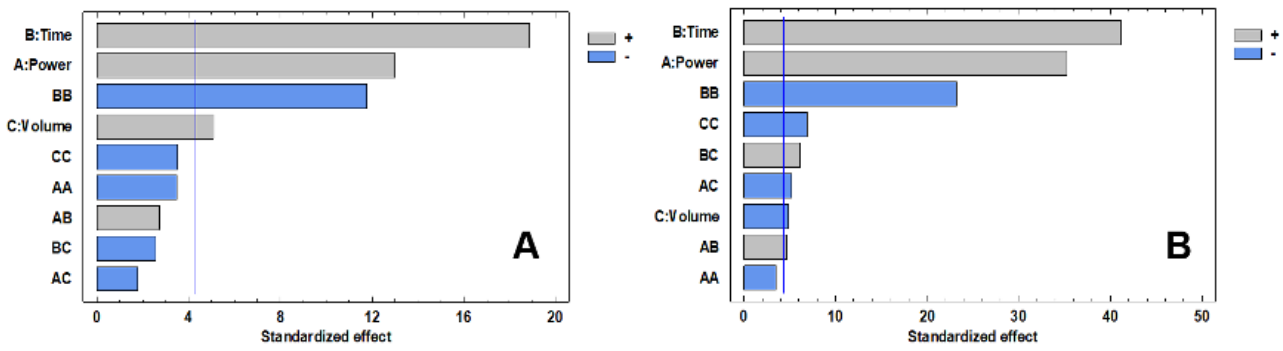

Figure 1. Standardized effects of MAHD EO extraction variables for **A)** *R. lingulata* aerial parts and **B)** rhizomes. Bar colors denote positive and negative direction effects. The Pareto diagram shows that the linear terms time (A) and power (B) and the quadratic factor time (B2), have greater effect on the MAHD.

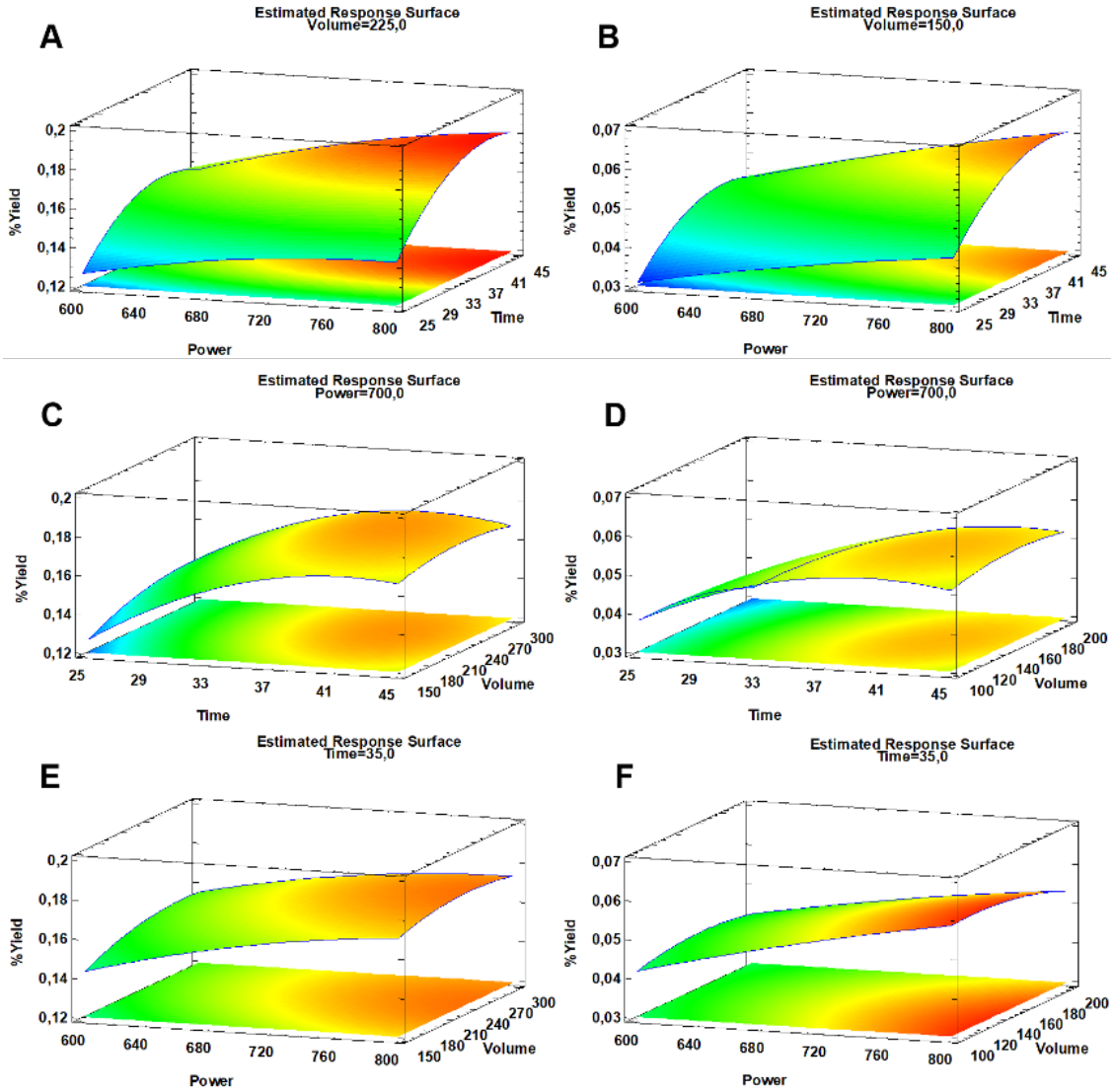

Figure 2. Response surface estimates of the MAHD process variables tested for EO extraction from *R. lingulata* aerial parts (A, C, and E) and rhizomes (B, D, and F).

Lastly, addressing the interaction between microwave radiation power and solvent volume for plant aerial parts and rhizomes, figures 2E and 2F respectively revealed that high radiation power affects positively EO yield, and that increasing solvent amount counters EO extraction yields, likely because the upper microwave power tests was not sufficient to promote and accelerate the effect of the mass transfer [19].

3.3. Optimal EO extraction parameters

EO extraction parameter data were analyzed via statistical prediction thus narrowing the optimal conditions that maximize its yield, depending on the plant parts from which the EO were obtained, as shown in **Table 6**.

Table 6. Optimal conditions for MAHD-assisted essential oil extraction form *R. ligulata* parts.

Essential oil source	Extraction factor			
	Microwave power (W)	Microwave radiation length (min)	Solvent volume (mL)	EO % Yield (w/w)
Aerial part (30 g)	765	42.75	225.9	0.18
Rhizome (20 g)	799	42	145	0.061

Fig. 3 displays the value space in which the combination of two EO extraction parameters (microwave power and radiation time) maximizes the desirability function in the for each EO source, revealing the combination of factor values at which the optimum is achieved.

Bearing in mind the optimal parameters obtained, triplicates of optimized experiments were performed resulting in a yield improvement of 0.2186 % ± 0.0160 % for extractions from *R. ligulata* aerial parts and 0.071 % ± 0.023 % for extractions from plant rhizomes. These values were close to those predicted by the model. Besides, we demonstrated that *R. ligulata* aerial parts had an EO content three times higher than that of its rhizomes.

3.4. Ultrasound pretreatment efficiency comparison

To evaluate ultrasound treatment effects on plant material before conducting EO extractions with the MAHD method, experiments were conducted and compared with those from previously optimized EO extractions. Results are shown in **Fig.4**.

Ultrasound pretreatment improved yield by 12.8 % in *R. ligulata* aerial parts and by 33.3 % in *R. ligulata* rhizomes. Such contrast is likely due to the mechanical effect of ultrasound, accelerating the release of the EO through a physical process called cavitation. The effects of caviation can induce different alterations in plant tissues, such as matrix fragmentation, cell erosion, pore formation, increased absorption, shear force, and changes in swelling index. Cavitation produces shock waves, micro-jets, shear force, and turbulence, modifying plant matrix and accelerating EO extraction [22, 23, 24, 25].

The GC-MS analysis of the obtained *R. ligulata* EOs led to the identification of their constituent metabolites revealing component variation, which is likely dependent employed plant part, as comprehensively shown in **Table 7** and the chromatograms in **Fig.5**.

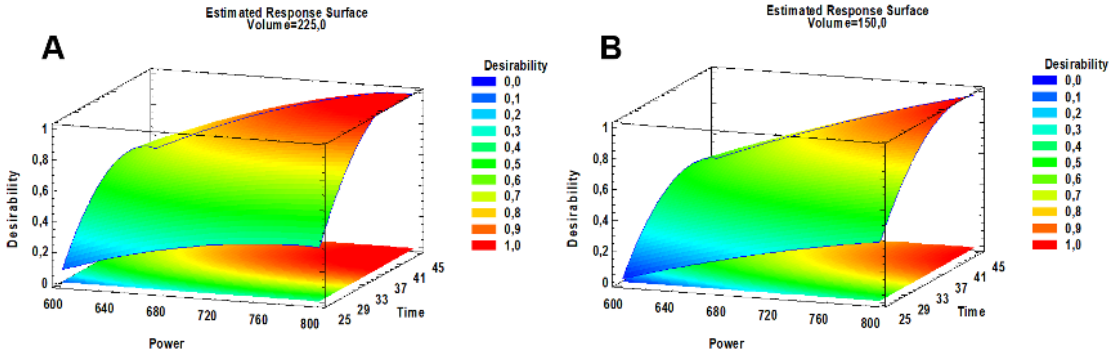

Figure 3. Response surface, Optimization A) AP, and B) Ri.

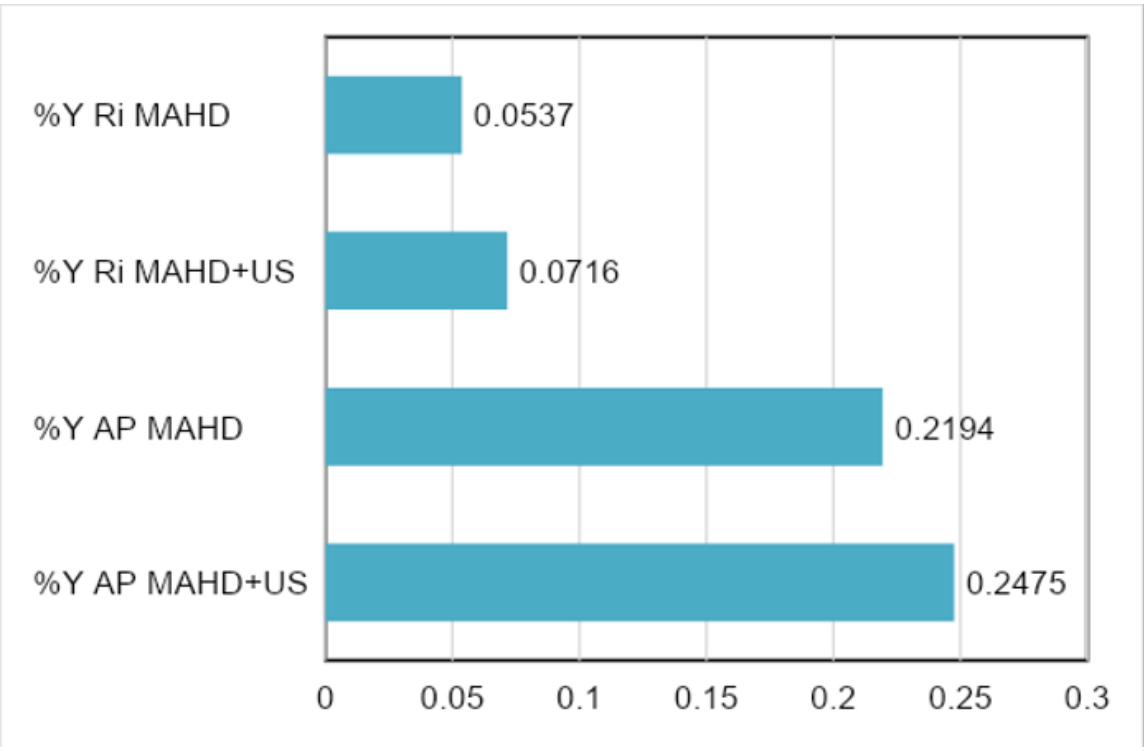

Figure 4. Essential oil yield percentages with and without ultrasound (US) treatment before MAHD extraction from *R. ligulata* rhizomes (Ri) and aerial parts (AP).

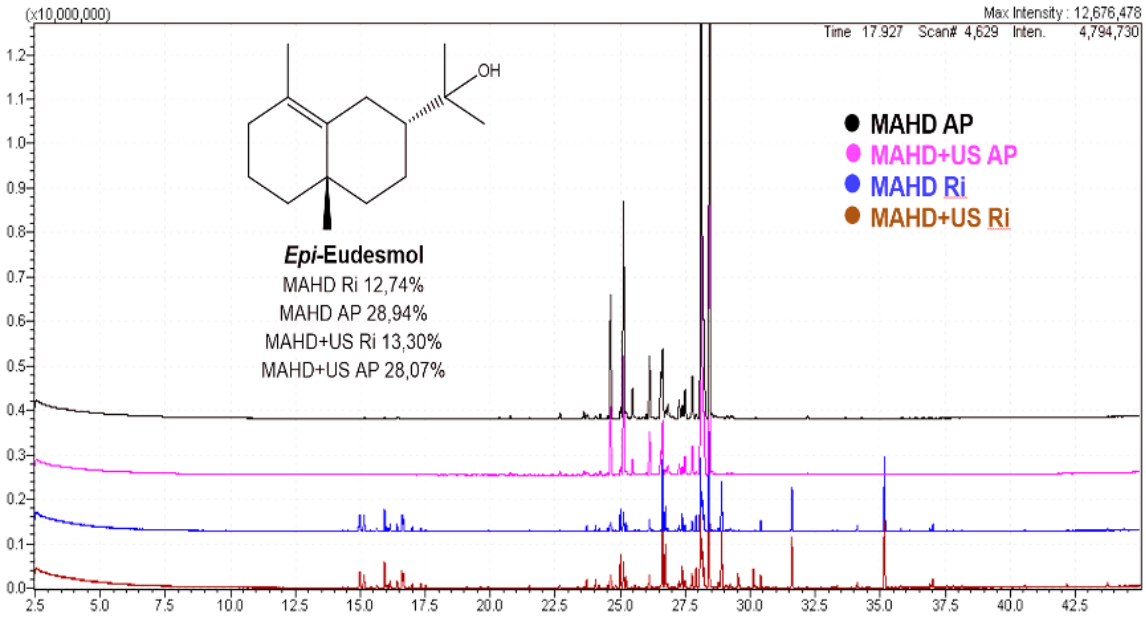

Figure 5. GC/MS chromatogram of *R. Ligulata* essential oil and with the chemical structure of the dominant compound given.

Table 7. Chemical composition of EOs.

				Relative area (%)			
Tr min	Compound name	RI (exp)	RI (tr)	MAHD-AP	MAHD +US AP	MAHD-Ri	MAHD +USRi
14.3	Dehydro sabinene ketone	1122	1117	NA	NA	NA	0.1
14.5	Canholenal	1129	1126	NA	NA	0.1	0.2
14.9	Sabinol	1144	1140	NA	NA	T1.8	2.1
15.1	Verbenol	1149	1141	NA	NA	1.4	2.0
15.4	2-Methyl-1-phenyl-2-propanol	1158	1158	NA	NA	0.1	0.1
15.6	Pinocarvone	1165	1164	NA	NA	0.18	0.41
15.9	Terpinen-4-ol	1176	1177	NA	NA	2.6	2.7
16.0	Isoverbanol	1179	1179	NA	NA	0.3	0.3
16.1	Verbanol	1183	1184	NA	NA	0.6	0.8
16.4	Dihydrocarvone	1191	1192	NA	NA	0.8	1.0
16.5	γ-Terpineol	1198	1199	NA	NA	2.0	2.2
16.6	(-)-Verbenone	1200	1205	NA	NA	1.3	1.4
16.9	Dihydrocarveol	1212	1214	NA	NA	0.4	0.5
17.3	Citronellol	1224	1225	NA	NA	0.4	0.4
17.5	Isocarveol	1231	1231	NA	NA	0.3	0.2
21.5	Isoledene	1377	1376	NA	NA	0.2	NA
23.7	Aromadendrene	1462	1462	NA	NA	0.8	0.6
23.7	Cadin-1(6),4-diene	1463	1463	0.3	0.3	NA	NA
24.0	Isocadinene	1476	1476	NA	NA	0.8	0.6
24.1	ar-Curcumene	1481	1480	NA	NA	0.2	0.2
24.5	α-Zingiberene	1494	1493	NA	NA	0.2	0.3
24.6	4-epi-Dihydroagarofuran	1499	1499	5.6	6.3	1.6	1.6
24.8	α-Bisabolene	1507	1507	NA	NA	0.2	0.16
25.0	γ-Cadinene	1514	1516	NA	NA	3.0	2.6
25.1	β-Curcumene	1515	1515	0.6	0.5	NA	NA
25.1	Dihydroagarofuran	1519	1523	9.8	11.0	2.6	2.5
25.2	Macrocarpene	1524	1527	0.35	0.3	1.10	1.2
25.4	10-epi-Cubebol	1534	1534	1.2	1.5	NA	NA
25.5	α-Cadinene	1538	1538	NA	NA	0.3	0.2
25.7	α-Calacoreneo	1544	1545	NA	NA	0.1	0.12
26.1	Nerolidol	1561	1563	3.3	3.0	1.3	1.3
26.5	Sesquisabinene hydrate	1580	1580	1.7	2.3	NA	NA
26.6	Caryophyllene oxide	1582	1583	4.5	3.7	9.1	8.6
26.7	Thujopsan-2-ol	1587	1587	0.3	0.3	4.4	3
26.8	Viridiflorol	1591	1592	0.6	0.5	NA	NA
27.2	β-Atlanton	1610	1608	1.0	1.0	0.5	0.4
27.3	Bisabolol oxide	1615	1616	0.7	0.7	2.4	2.1
27.4	Cedrol	1619	1619	1.5	1.5	0.7	0.8
27.7	γ-Eudesmol	1632	1632	2.4	2.2	1.6	1.5

27.9	epi-Cadinol	1639	1640	NA	NA	2.1	2.2
28.0	α-Muurolol	1647	1646	36.8	36.2	8.9	9.1
28.1	β-Eudesmol	1649	1650	NA	NA	2.8	2.8
28.2	α-Eudesmol	1652	1653	NA	NA	1.7	1.9
28.4	epi Eudesmol	1661	1662	28.9	28.0	12.7	13.3
28.7	Cadalene	1677	1676	NA	NA	0.6	0.5
28.8	α-Bisabolol	1683	1685	NA	NA	5.7	6.3
29.2	Nootkatol	1698	1699	NA	NA	0.3	0.3
29.5	Farnesol	1712	1715	NA	NA	1.1	NA
30.1	Santalol	1739	1739	NA	NA	1.8	5.5
30.4	Nootkatona	1808	1806	NA	NA	0.6	0.6
31.5	Eudesm-11-en-4-α, 6-α-diol	1846	1844	NA	NA	8.2	9.2
34.1	ent-Rosa-5,15-diene	1934	1934	NA	NA	0.6	0.6
35.1	Pseudophytol	1988	1988	NA	NA	8.2	9.2
36.9	Frankincense oxide	2082	2080	NA	NA	0.3	0.4

	Structural compound family	**MAHD-AP**	**MAHD +US AP**	**MAHD-Ri**	**MAHD+US Ri**
	Monoterpene hydrocarbons	0.0	0.0	1.6	2.0
	Oxygenate monoterpene	0.0	0.0	10.2	9.6
	Sesquiterpene hydrocarbons	8.4	7.6	4.3	5.2
	Oxygenate sesquiterpene	86.3	85.6	77.3	81.1
	Others	5.3	6.8	18.4	13.7

The compound epi-Eudesmol was the dominant component of EO in *R. Ligulata* aerial parts and rhizomes (Table 7 and in Figure 5). This result is of interest, because this molecule is considered a neuroprotector with potential pharmacological, some studies on EO rich in α-Muurolol and epi-Eudesmol have shown a powerful bactericidal effect on *Escherichia coli* and *Salmonella typhimirium*, opening a window for studies of its biological activity and future applications [13]. Furthermore, to the best of our knowledge, ours is the first report on the optimization, extraction, and metabolic characterization of EO of *R. Ligulata*.

Regarding the chemical composition of the obtained *R. Ligulata* EO, monoterpenes constituted 11 % of the extract with and without US pretreatment on plant rhizomes; whereas, aerial parts did not contained monoterpenes. Furthermore, the main structural family in EO from aerial parts and rhizomes were sesquiterpenes with areas from 80 % to 90 %, depending on the plant organ and the extraction method. Likewise, it was possible to verify that the main components of EO from from aerial parts and rhizomes were Caryophyllene oxide, α-Muurolol, and epi-Eudesmol.

4. Conclusions

The MAHD essential oil extraction parameters with the best response surface optimization results were a microwave power of 765 W and a solvent volume of 225.9 mL for 30 g of *R. Ligulata* aerial parts and a microwave power of 799 W and 145 mL of solvent for for 20 g of *R. Ligulata* rhizomes. The best average extraction time was 42.5 min.

The results obtained herein reveled the utility of ultrasound pretreatment on plant material before MAHD essential oil extraction. The cavitation effect of ultrasound increased the release of essential oils by 12.8 % in *R. Ligulata* aerial parts and by 33.3 % in rhizomes.

The dominant compound in the MAHD extracted *R. Ligulata* essential oils was epi-Eudesmol, with 28 % in aerial parts and 13 % in rhizomes. This is an interesting neuroprotective molecule that inhibits calcium channels.

This work highlights the importance of continuing the search for different plant metabolites, with potential pharmacological applications that positively impact public health, via novel extraction and synthesis methods with improved efficiencies and minimal environmental burdens.

5. Conflict of interests

The authors have no conflict of interest to declare.

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Optimización de un nuevo método de extracción de aceite esencial de *Renealmia ligulata* (Zingiberaceae) mediante hidrodestilación asistida por microondas

Resumen: *Renealmia* es un género de plantas tropicales perteneciente a la familia Zingiberaceae. En la región tropical de América del Sur, las plantas de *Renealmia* son conocidas por sus usos terapéuticos para combatir dolores óseos y musculares, así como los resfriados y para contrarrestar los síntomas de la mordedura de serpiente. A pesar de la importancia biomédica de los metabolitos de *Renealmia*, los componentes de sus aceites esenciales (AE) han sido escasamente estudiados. En parte, esto se debe a la falta de métodos locales para extraer aceites esenciales que sean eficientes, económicos y respetuosos con el medio ambiente. Este trabajo abordó la optimización de un método de extracción de AE a partir de las partes aéreas y rizomas de plantas de *Renealmia ligulata* basado en hidrodestilación asistida por microondas (MAHD) con un pretratamiento de extracción asistida por ultrasonidos (EAU). Se estudiaron tres variables de extracción de MAHD por sí solas y en combinación (potencia de radiación, duración de la exposición a la radiación y volumen de solvente). Realizamos un análisis de superficie de respuesta para determinar las combinaciones de valores que resultaban en rendimientos óptimos de AE. Los resultados indicaron que el mejor tiempo promedio para la extracción fue de 42,5 minutos, combinado con una potencia de radiación de 765 W y un volumen de solvente de 225,9 mL para 30 g de material vegetal de partes aéreas o 799 W y 145 mL de solvente para 20 g de rizomas vegetales. Un análisis GC-MS de los AE de *R. ligulata* obtenidos reveló que su principal componente era epi-Eudesmol (28% en las partes aéreas de la planta y 13% en los rizomas), que es una molécula de interés debido a sus propiedades neuroprotectoras reportadas.

Palabras Clave: Epi-Eudesmol; Aceites esenciales; Hidrodestilación asistida por microondas; *Renealmia*, Análisis de la superficie de respuesta; Asistido por ultrasonido.

Otimização de um Novo Método de Extração de Óleo Essencial de *Renealmia ligulata* (Zingiberaceae) por Hidrodestilação Assistida por Micro-ondas

Resumo: *Renealmia* é um género de plantas tropicais pertencente à família Zingiberaceae. Na região tropical da América do Sul, as plantas de *Renealmia* são conhecidas por seus usos terapêuticos para combater tanto dores ósseas e musculares como resfriados e para neutralizar sintomas de picadas de cobra. Apesar da importância biomédica dos metabólitos da *Renealmia*, os componentes de seus óleos essenciais (OE) têm sido pouco estudados. Em parte, isso se deve à falta de métodos locais de extração de óleos essenciais que sejam eficientes, econômicos e ecologicamente corretos. Este trabalho abordou a otimização de um método de extração de OE das partes aéreas e rizomas de plantas de *Renealmia* ligulata baseado em hidrodestilação assistida por micro-ondas (MAHD) com um pré-tratamento de extração assistida por ultrassom (EAU). Estudamos três variáveis de extração de MAHD isoladamente e em combinação (potência da radiação, duração da exposição à radiação e volume do solvente). Realizamos uma análise de superfície de resposta para identificar as combinações de valores que resultaram em desempenhos ótimos de extração de OE. Os resultados indicaram que o melhor tempo médio para a extração foi de 42,5 minutos, combinado com uma potência de radiação de 765 W e um volume de solvente de 225,9 mL para 30 g de material vegetal das partes aéreas ou 799 W e 145 mL de solvente para 20 g de rizomas vegetais. Uma análise por CG-MS dos OE de *R. ligulata* obtidos revelou que seu principal componente era o epi-Eudesmol (28% nas partes aéreas da planta e 13% nos rizomas), que é uma molécula de interesse devido às suas propriedades neuroprotetoras relatadas.

Palavras-chave: Epi-Eudesmol; Óleos essenciais; Hidrodestilação assistida por micro-ondas; *Renealmia*; Análise de superfície de resposta; Assistido por ultrassom.

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