plants

MDPI

Article

Oral and Topical Anti-Inflammatory Activity of *Jatropha integerrima* Leaves Extract in Relation to Its Metabolite Profile

Engy A. Mahrous [1,*], Ahmed H. Elosaily [2], Abeer A. A. Salama [3], Ahmed M. Salama [2] and Soheir M. El-Zalabani [1]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt; soheir.elzalabani@pharma.cu.edu.eg
[2] Department of Pharmacognosy, Faculty of Pharmacy, Ahram Canadian University, Giza 12573, Egypt; elosaily1987@hotmail.com (A.H.E.); am.salama@hotmail.com (A.M.S.)
[3] Pharmacology Department, National Research Center, Cairo 12622, Egypt; berrotec@yahoo.com
* Correspondence: engy.abdelhamid@pharma.cu.edu.eg

Abstract: *Jatropha integerrima* Jacq., family: Euphorbiaceae, is used in India and subtropical Africa to treat different skin conditions. In this study we evaluated the anti-inflammatory activity of *J. integerrima* leaves extract (JILE) using rat paw edema model. The extract was administered orally (200 and 400 mg/kg) or applied topically as creams at 2.5, 5, and 10% strength. Four hours post-treatment, maximum reduction of edema volume by 63.09% was observed after oral administration of JILE (400 mg/kg) as compared to indomethacin with 60.43%. The extract anti-inflammatory effect was accompanied by a decrease in NO, prostaglandin PGE2, TNF-α and PKC levels by 19, 29.35, 16.9, and 47.83%, respectively. Additionally, topical applications of JILE showed dose dependent reduction in paw edema and resulted in normalized levels of PGE2, TNF-α, and PKC when used as 10% cream. Signs of inflammations were reduced or absent from paw tissue of animals receiving JILE either orally or topically. Finally, liquid chromatography/mass spectrometry analysis of JILE resulted in the annotation of 133 metabolites including 24 diterpenoids, 19 flavonoids, 10 phenolic acid conjugates, 8 cyclic peptides, 6 phytosterols, 4 sesquiterpenes, and 4 coumarins. Several of the annotated metabolites have known anti-inflammatory activity including vitexin, isovitexin, fraxitin, scopeltin, stigmasterol, and many diterpenoidal derivatives.

Keywords: anti-inflammatory; diterpenoids; cyclic peptide; Euphorbiaceae; hydroxyl fatty acids *Jatropha integerrima*; vitexin; UPLC/MS-MS

Citation: Mahrous, E.A.; Elosaily, A.H.; Salama, A.A.A.; Salama, A.M.; El-Zalabani, S.M. Oral and Topical Anti-Inflammatory Activity of *Jatropha integerrima* Leaves Extract in Relation to Its Metabolite Profile. *Plants* **2022**, *11*, 218. https://doi.org/10.3390/plants11020218

Academic Editors: Yun-Soo Seo and Joong-Sun Kim

Received: 30 November 2021
Accepted: 10 January 2022
Published: 14 January 2022

1. Introduction

The genus *Jatropha* (family Euphorbiaceae) has a wide distribution in tropical and subtropical regions especially South America, West Africa, India, and Southeast Asia [1,2]. Many members of the genus were used medicinally in their indigenous countries such as *J. gossypifolia*, *J. curcas*, *J. chevalieri*, and *J. multifida* [3]. Leaves and latex from *Jatropha* plants are especially useful in treating skin conditions such as ulcers, blisters, eczema and also to accelerate wound healing [4–6]. The genus is rich in bioactive secondary metabolites especially diterpenoids of tigliane, lathyrane, and jatrophane skeletons which exist mainly as esters [7,8]. However, among more than 175 *Jatropha* species, only few species were chemically investigated. As a result of these investigations, many structurally unique and bioactive phytochemicals were identified including flavonoids, cyclic peptides, lignans, and diterpenes [1,3].

Jatropha integerrima Jacq (*syn. Jatropha pandurifolia* And.), also known as spicy jatropha, is cultivated around the world as an ornamental shrub due to its showy bright red flowers. Its leaves are used as poultice in India and Bangladesh to treat different conditions including eczema, pruritus and skin warts [9,10]. Phytochemical investigation of *J. integerrima* identified coumarins [11], cyclic peptides [12], neolignans [13], and several novel diterpenes

with different biological activities [14,15]. More than 16 new compounds were isolated from *J. integerrima* in the past decade alone [13–15], indicating a rich metabolome that is yet to be fully explored. Recent pharmacological investigation of the plant showed promising antimicrobial activity of extracts and essential oils obtained from seeds and leaves [16,17] and strong antioxidant activity of the flowers extract [18].

Based on the accumulated literature and the known folk use of *J. integerrima*, this study was performed to investigate the anti-inflammatory effect of *J. integerrima* leaves extract (JILE) when administered orally or applied topically. Anti-inflammatory activity was assessed in rat paw edema model through measuring edema volumes and levels of inflammatory mediators nitric oxide (NO), tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2), and protein kinase C (PKC) in addition to examining histopathological features of paw tissue. Furthermore, ultra-performance liquid chromatography coupled to high resolution mass spectrometry (UPLC-MS) was used to identify secondary metabolites in JILE through tandem mass fragmentation to assist with identifying metabolites that can contribute to the anti-inflammatory activity of the plant.

2. Results

2.1. Acute Toxicity Study

Rats treated with *J. integerrima* leaves extract (JILE) at a high dose of 5 g/kg did not show any skin abnormalities or changes in respiratory, circulatory, and somatomotor activities as well as behavior pattern.

2.2. Evaluation of Oral Anti-Inflammatory Effect of Jatropha integerrima Leaves Extract

The subplanter injection of 100 µL of 1% sterile carrageenan into the rat hind paw elicited an inflammation manifested by swelling, erythema and a time-dependent increase in paw edema by 61.82, 77.58, 87.24%, and 66.37% after 1, 2, 3, and 4 h after injection, as compared with precarrageenan control values, Figure 1. This observed response to carrageenan injection follows the same pattern of maximum inflammation after 3 h as previously reported [19].

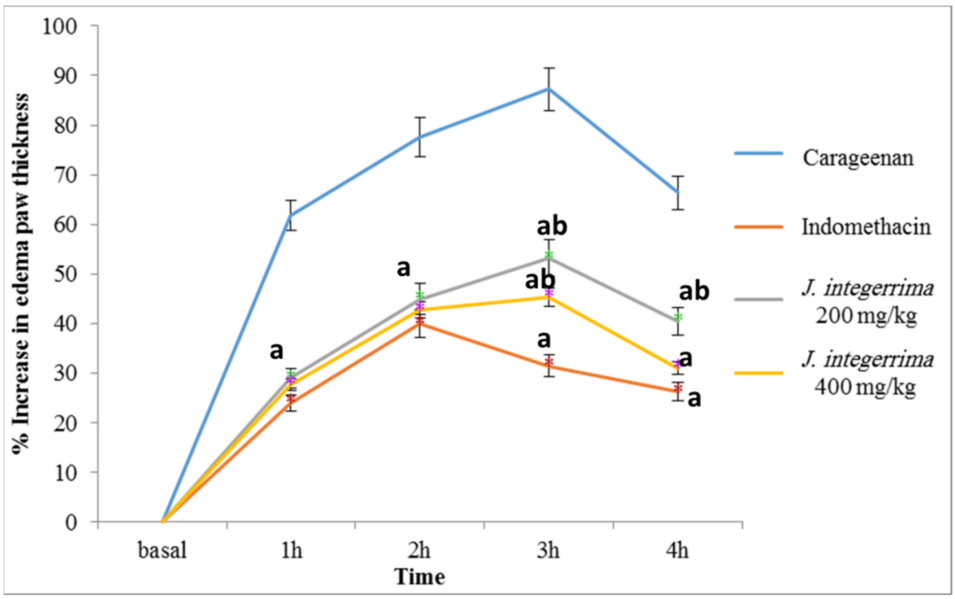

Figure 1. Effect of oral administration of ethanol extract of *Jatropha integerrima* leaves on edema volume. One way ANOVA and Fishers LSD comparison test were used, $p < 0.05$; a: significantly different from carrageenan control group at respective time point; b: significantly different from indomethacin group at respective time point.

Pretreatment with JILE (200 mg/kg and 400 mg/kg, orally) showed a significant inhibition of edema formation by 53.09% and 55.17% after 1 h, 42.15% and 44.87%, after 2 h, 39.04% and 48.03% after 3 h, and 39.01% and 63.90% after 4 h, respectively, as compared with carrageenan control group at the same time. In the same assay, indomethacin treatment (25 mg/kg) resulted in a 61.17%, 48.56%, 63.9%, and 60.43% reduction of edema volume, as compared with that of carrageenan group after 1, 2, 3, and 4 h postcarrageenan injection, respectively, Figure 1.

By the end of the experiment, serum NO level increased by 44% in animals that received only carrageenan as compared with normal control group. Animals that were pretreated with indomethacin and *J. integerrima* at 200 mg/kg and 400 mg/kg had significantly decreased serum NO levels by 17%, 13%, and 19%, respectively, as compared with that of animals that received carrageenan only ($p < 0.05$), Table 1.

Similarly, after 4 h following carrageenan injection, serum levels of inflammation mediators such as PGE2, TNF-α, and PKC were elevated by 98.5%, 38.75%, and 72.94%, respectively, as compared with that of normal control group. Animals pretreated with indomethacin had their levels of PGE2 decreased by 28.85%, TNF-α by 17.76%, and PKC by 44%. Meanwhile, pretreatment with JILE at 200 mg/kg caused insignificant changes in serum levels of TNF-α and PGE2 ($p > 0.05$) but reduced PKC by 46.47%, Table 1. Meanwhile, pretreatment with JILE at a dose of 400 mg/kg decreased PGE2 by 29.35%, TNF-α by 16.90% and PKC by 47.83%, as compared with carrageenan control group resulting in restoring serum levels of NO and PKC to their basal levels and were not significantly different from the effect observed with indomethacin treatment ($p > 0.05$).

The paw tissue in the healthy group and the group treated with carrageenan and indomethacin displayed normal histological features (Figure 2a,b), while the carrageenan treated group showed a few inflammatory cells infiltration in the dermal tissue and massive inflammatory cell infiltration in the subcutaneous tissue (Figure 2c). Mild focal inflammatory aggregation in subcutaneous tissue was noticed in the group treated with JILE (200 mg/kg) with normal dermal tissue (Figure 2d) while the dermal and subcutaneous tissues appeared intact in the group treated with JILE at 400 mg/kg, (Figure 2e).

Table 1. Effect of oral administration of *Jatropha integerrima* leaves extract (JILE) on different inflammation biomarkers.

	Control	**Carrageenan**	**Indomethacin 25 mg/kg**	**JILE 200 mg/kg**	**JILE 400 mg/kg**
NO (μmol/L)	16.00 ± 1.29	23.00 ± 0.676 [a]	19.03 ± 0.3 [ab]	19.9 ± 0.08 [ab]	18.55 ± 0.39 [b]
PGE2 (pg/mL)	251.28 ± 4.16	498.8 ± 37.04 [a]	354.88 ± 5.36[ab]	514.88 ± 5.36 [a]	352.4 ± 23.52 [ab]
TNF-α (pg/mL)	1581.92 ± 10.88	2194.56 ± 99.52 [a]	1804.8 ± 7.84 [ab]	2104.96 ± 4.16 [a]	1823.68 ±0.16 [ab]
PKC (pg/mL)	870.4 ± 99.705	1505.35 ± 5.78 [a]	842.35 ± 153.85 [b]	805.8 ± 62.985 [b]	785.4 ± 23.29 [b]

Data were expressed as mean ± SD. Statistical analysis was carried out by one-way ANOVA, followed by Tukey's HSD test for multiple comparisons. [a]: significantly different from normal control (Saline), [b]: significantly different from carrageenan control at $p < 0.05$.

2.3. Evaluation of Topical Anti-Inflammatory Activity of Jatropha integerrima Leaves Extract

Rats given *J. integerrima* cream in the skin irritation test showed no sensitivity or irritation. The subplantar injection of carrageenan into the rat hind paw elicited an inflammation as previously mentioned in Section 2.2. Topical application of base non-medicated cream and 2.5% strength JILE cream before carrageenan injection showed no significant inhibition of edema formation at all-time points as compared with that of carrageenan group, Figure 3. Meanwhile topical application of higher strength JILE creams (5% and 10%) showed a significant inhibition of edema formation at all-time points especially after 4 h by 58.27% and 68.96%, respectively, as compared with that of untreated animals. In addition, the effect

produced upon application of 10% JILE cream was superior to standard hydrocortisone cream ($p < 0.05$), Figure 3.

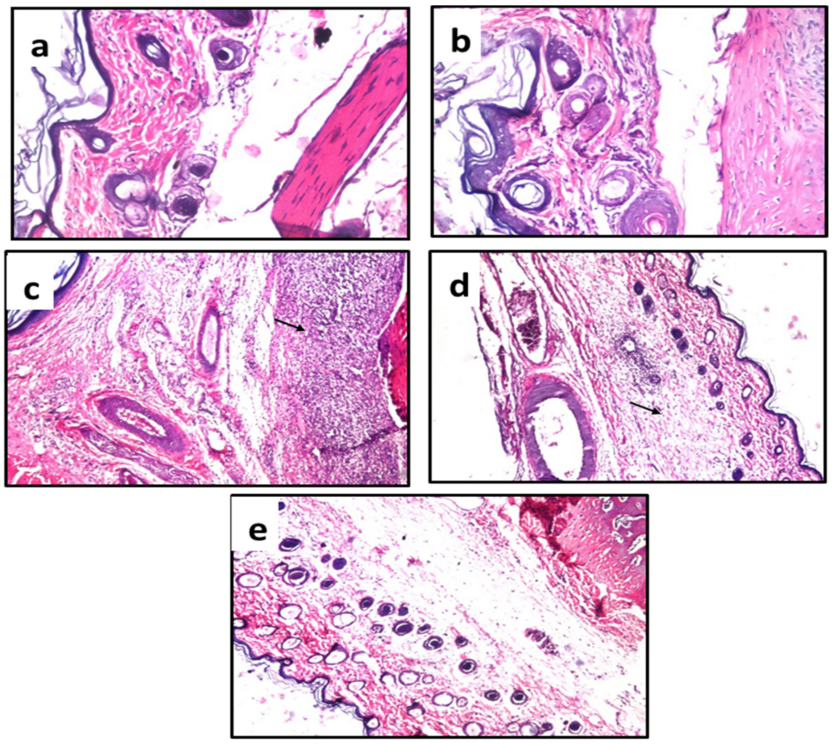

Figure 2. Photomicrographs of subcutaneous and dermal tissues of treated rats. (**a**) control group; (**b**) indomethacin group; (**c**) carrageenan group; (**d**) group receiving oral 200 mg/kg of *J. integerrima* leaves extract; and (**e**) group receiving oral 400 mg/kg of *J. integerrima* leaves extract. Arrows refer to inflammatory cell infiltration.

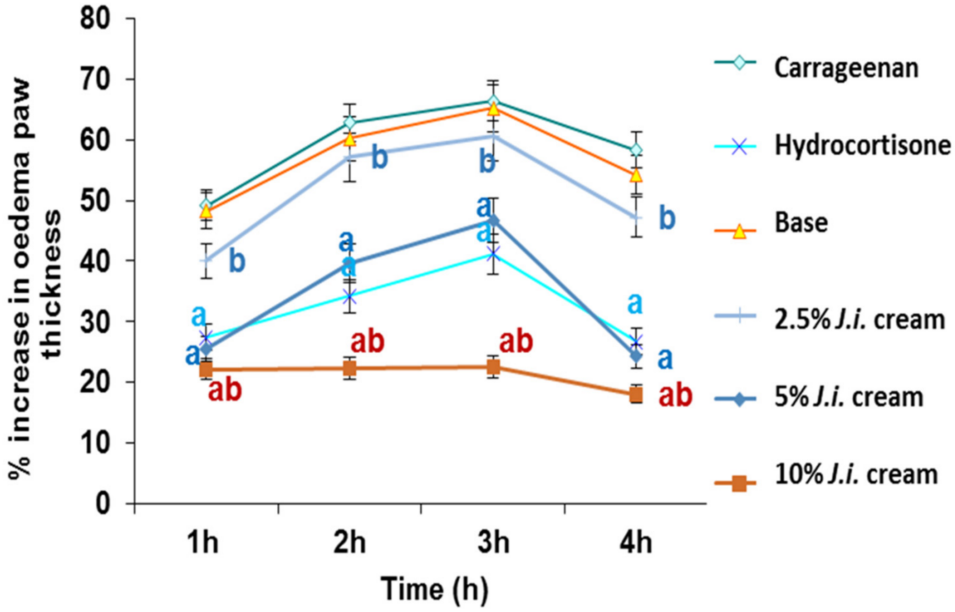

Figure 3. Effect of topical administration of *Jatropha integerrima* cream on edema volume in rat hind paw model. One way ANOVA and Fishers LSD comparison test* were used in data analysis, $p < 0.05$; a significantly different from carrageenan control value at respective time point. b significantly different from hydrocortisone group value at respective time point.

Four hours postcarrageenan injection, inflammatory mediators were measured. An increased serum NO levels by 39.66% and 35.81% were observed in carrageenan treated animals and in animals treated with base cream, respectively, as compared with that of healthy control group. Animals that were treated with hydrocortisone cream and JILE cream at 2.5%, 5% and 10% showed significantly decreased serum NO levels by 14.84%, 10.92%, 17.04%, and 18.04%, respectively, as compared with that of untreated animals, Table 2.

As expected for animals receiving carrageenan only or treated with base cream, levels of inflammatory mediators were all elevated. An increase of serum levels by 32.86% and 32.03% in case of PGE2, 21.89% and 18.95% for TNF-α, and 47.37% and 46.43% in case of PKC were observed in animals that received no treatment or base cream only, respectively. Treatment with hydrocortisone resulted in a decrease in serum levels of PGE2 by 21.94%, TNF-α by 15.12%, and PKC by 32.13% when compared with that of untreated group. Meanwhile, pretreatment with cream containing 2.5% of JILE decreased PGE2 and PKC levels by 10.41% and 23.32%, respectively, while animals treated with cream containing 5% JILE decreased PGE2, TNF-α and PKC by 14.95%, 6.51%, and 26.58%, respectively, as compared with that of carrageenan group. Meanwhile, animals that received cream containing 10% of extract showed normalized serum levels of TNF-α, PKC, and PGE2, as compared with that of control group, as did treatment with hydrocortisone cream, Table 2.

Table 2. Effect of topical administration of ethanol extract of *Jatropha integerrima* cream on different inflammation biomarkers.

	Control	Carrageenan	Hydrocortisone 1% Cream	Base	JILE 2.5% Cream	JILE 5% Cream	JILE 10% Cream
NO (μmol/L)	17.9 ± 0.7	25 ± 0.7 [a]	21.29 ± 0.6 [ab]	24.31 ± 0.1 [a]	22.27 ± 0.59 [ab]	20.74 ± 0.58 [ab]	20.49 ± 0.08 [ab]
PGE2 (pg/mL)	215.4 ± 1.5	286.2 ± 0.4 [a]	223.4 ± 0.2 [b]	284.4 ± 1.8 [a]	256.4 ± 1.4 [ab]	243.4 ± 1.1 [ab]	216.4 ± 1.1 [b]
TNF-α (pg/mL)	1561.9 ± 13.6	1903.9 ± 62.9 [a]	1615.9 ± 15.68 [b]	1857.9 ± 78.1 [a]	1804.5 ± 23.7 [a]	1779.9 ± 4.4 [ab]	1609.9 ± 22.3 [b]
PKC (pg/mL)	854.9 ± 22.8	1259.9 ± 4.4 [a]	854.98 ± 15.1 [b]	1251.9 ± 26.4 [a]	965.98 ± 9.8 [ab]	924.9 ± 11.1 [ab]	839.9 ± 2.2 [b]

Data were expressed as mean ± SD. Statistical analysis was carried out by one-way ANOVA followed by Tukey's HSD test for multiple comparisons. [a]: Significantly different from normal control (Saline) at $p < 0.05$. [b]: Significantly different from carrageenan control at $p < 0.05$.

The paw tissue in the healthy group and group treated with carrageenan and hydrocortisone displayed normal histological features (Figure 4a,c), except for few inflammatory cells in the hydrocortisone group. Animals that were injected with carrageenan but received no further treatment showed massive inflammatory cell infiltration in the subcutaneous tissue (Figure 4b). Hemorrhages, inflammatory cells, and hyalinization were noticed in animals treated with base cream only (Figure 4d). Edema with inflammatory cells infiltration was detected in animals treated with creams containing 2.5% and 5% of JILE (Figure 4e,f), while animals treated with 10% JILE cream showed few inflammatory cells in subcutaneous tissue, and the skin layer remained intact, Figure 4g.

2.4. UPLC-MS Analysis of Jatropha integerrima Extract

A total of 133 compounds were annotated in the extract representing different primary and secondary metabolites. Flavonoids and amino acids were eluted between 0–6 min, cyclic peptides were eluted from 9–11 min while diterpenoids, sterols, fatty acids, and fatty acid glycerides were eluted at the late part of the chromatographic run, Figure 5.

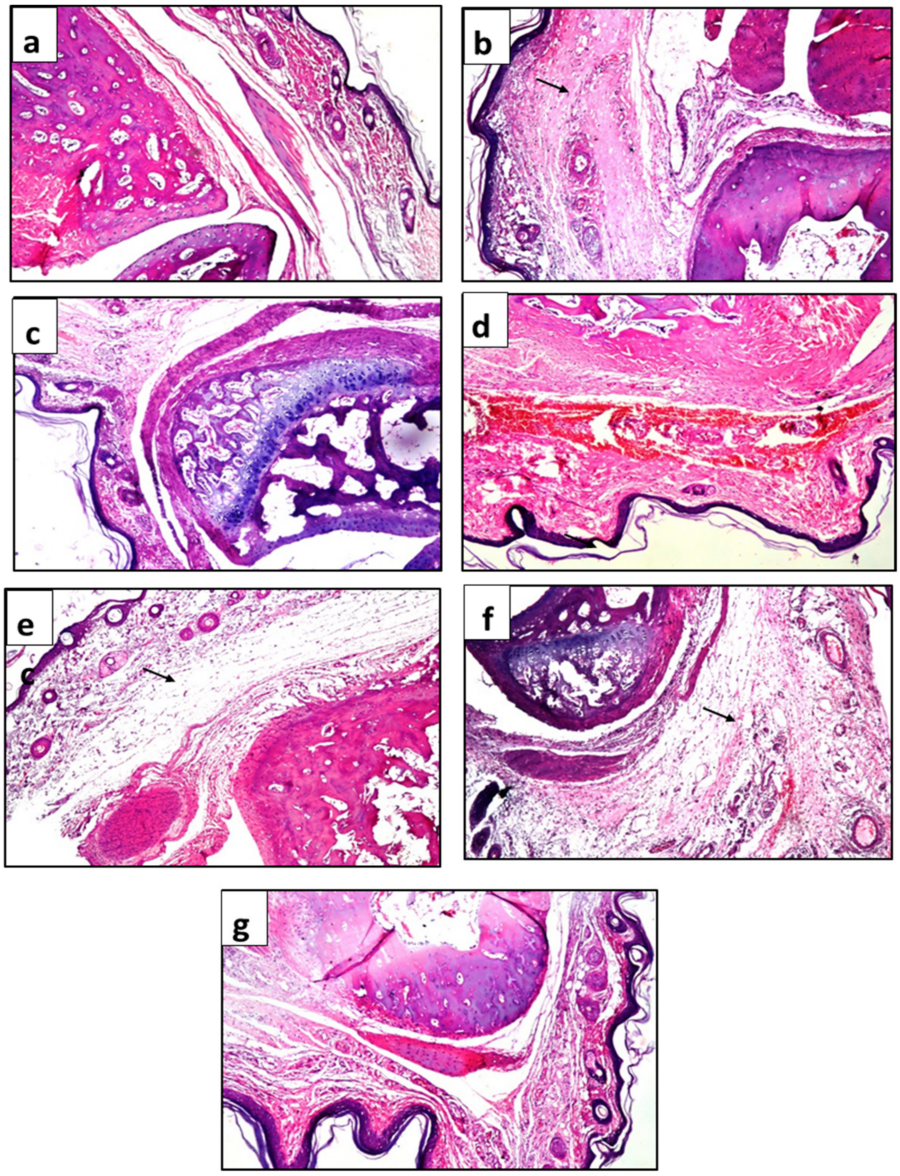

Figure 4. Photomicrographs of subcutaneous and dermal tissues of treated rats. (**a**) healthy animals; (**b**) animals treated with carrageenan only; (**c**) 1% hydrocortisone cream; (**d**) base cream; (**e**) 2.5% JILE cream; (**f**) 5% JILE cream; (**g**) 10% JILE cream. Arrows refer to inflammatory cell infiltration.

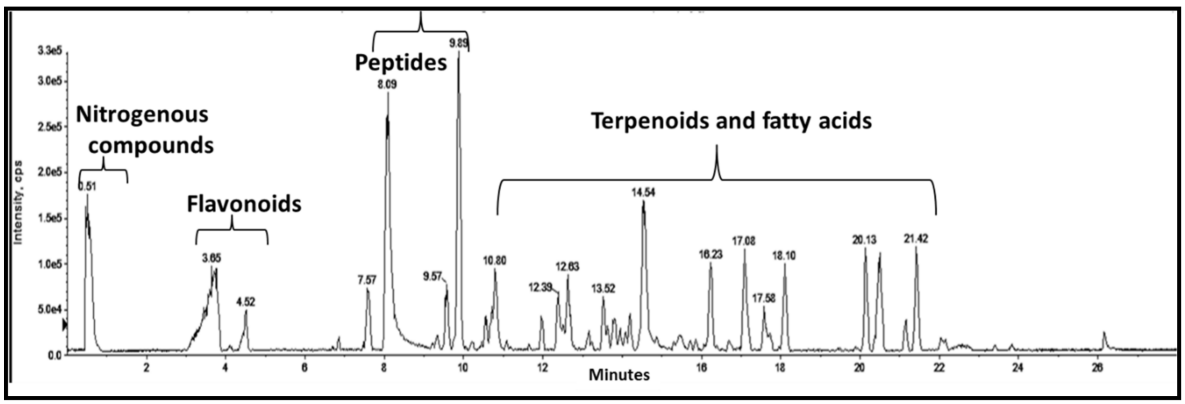

Figure 5. Base peak chromatogram of the UPLC/MS analysis of *J. integerrima* leaves extract.

2.4.1. Phenolic Compounds

Analysis of the UPLC/MS chromatogram resulted in the identification of 19 flavonoids, 10 phenolic acid conjugates, 4 coumarins, and 1 lignan. Flavonoids occurred mainly as *C*-linked hexoses (11 flavonoids) which were readily identified by the neutral loss of 120 Da, Figure S1, as observed in peaks 10, 11, 13, 14, 19, 22, 24, 29, 30, 32 and 35 (Table 3, Figure 6). Most of the *C*-glycosides were derivatives of apigenin, including isoorientin (peak 11), vitexin (peak 13), and isovitexin (peak 14), which are reported here for the first time from *J. integerrima*. Vitexin (C-8 glycoside) was differentiated from its structural isomer isovitexin (C-6) by the intensity of the fragment ions at m/z 313 and 283, produced due to the cleavage of the C-attached sugar, Figure S2.

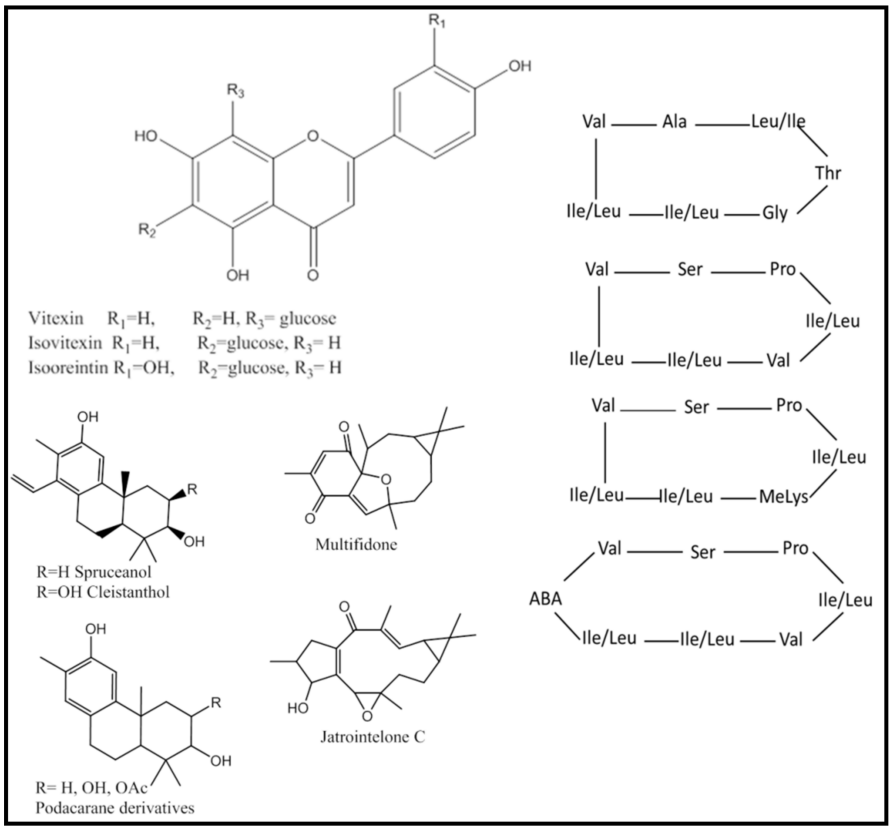

Figure 6. Structures of selected compounds annotated in *J. integerrima* extract including flavonoids, peptides, and diterpenoids.

Two *C*-glycosides dimers were observed at 5.25 and 5.94 min (peaks of 30 and 32, respectively) with $[M + H]^+$ at m/z 877.218 for $C_{30}H_{41}O_{20}$ and were assigned to the isomeric jatrophenols I/II/III previously isolated from *J. multifida* [20]; they were also detected in two other *Jatropha* species [21]. These are dimers of isovitexin connected by a methylene bridge [22], Figure S3. Fragmentation of these dimers resulted in loss of isovitexin monomer (−432 Da) and appearance of methylisovitexin fragment ion at m/z = 445, Table 3, Figure S3. Two other peaks (peaks 19 and 35) produced the same fragment ion (m/z = 445) with $[M+H]^+$ observed at m/z 560.1788 (for $C_{27}H_{30}NO_{12}$) and 736.2208 (for $C_{33}H_{37}NO_{15}$), respectively. Both peaks showed loss of proline amino acid as indicated by the neutral loss of 115 Da for $C_5H_9NO_2$, Figure S4 and S5 and produced methylisovitexin fragment ions at m/z = 445 and 427. Therefore, peak 19 and 35 were annotated as methylisovitexin proline and methylisovitexin proline ferulate. To the best of our knowledge, this is the first report of isovitexin proline derivatives.

Meanwhile, flavonoids *O*-glycosides were characterized by the neutral loss of 162 Da for hexoses (peaks 10, 16, 22, 26), Figure S6, and 146 for rhamnosyl residue (peak 25) [23], Figure S7, Table 3.

Phenolic acids in *J. integerrima* extract were detected only as conjugated molecules substituted with medium or long aliphatic chains, as seen in peaks 52, 56, 74, 76,106, 118, 128, and 129. Among the annotated phenolic acid esters, only tetradecyl ferulate (peak 118) was previously reported in the genus and was isolated from both *J. curcas* [24] and *J. multifida* [25].

Table 3. Annotated metabolites in leaves extract of *Jatropha integerrima* using UPLC/MS-Q-TOF analysis.

Peak No.	tR (min)	Experimental *m/z* $[M + H]^+$	Molecular Formula	Error (ppm)	Fragments (*m/z*)	Tentative Identification
			Flavonoids			
10	0.981	595.1656	$C_{27}H_{30}O_{15}$	3.4	577, 433, 379, 367, 313, 283	Isovitexin-*O*-hexoside
11	2.68	449.1076	$C_{21}H_{20}O_{11}$	2.3	431,383, 353, 339, 329, 299	Isoorientin
13	2.915	433.1116	$C_{21}H_{20}O_{10}$	3.2	415, 379, 367, 337, 313, 283	Vitexin
14	3.13	433.1116	$C_{21}H_{20}O_{10}$	3.2	415, 379, 337, 323, 313, 283	Isovitexin
16	3.4	449.1076	$C_{21}H_{20}O_{11}$	2.9	431, 395, 287	Kaempferol *O* hexoside
19	3.67	560.1788	$C_{27}H_{29}NO_{12}$	4.5	445, 427,409, 325, 295	Methylisovitexin proline
20	3.912	595.1672	$C_{27}H_{30}O_{15}$	2.2	287, 271	Kaempferol *O*-rutinoside
22	4.05	771.2151	$C_{37}H_{38}O_{18}$	2.6	675, 651,433,415, 313, 283, 177	Vitexin ferulate-*O*-hexoside
24	4.123	771.2111	$C_{37}H_{38}O_{18}$	−2.6	675, 651,433,415, 313, 283, 177	Isovitexin ferulate hexoside
25	4.253	579.1708	$C_{27}H_{30}O_{14}$	−0.4	433,417,399,351,321 297, 271	Apigenin *O*-rhamnoside-*O*-hexoside
26	4.396	433.1155	$C_{21}H_{20}O_{11}$	4.6	271	Apigenin-*O*-hexoside
27	4.44	579.1717	$C_{27}H_{30}O_{14}$	−1.5	271.0597	Apigenin-*O*-rutinoside
29	5.213	553.1329	$C_{28}H_{24}O_{12}$	−1	433, 415,397,337,313, 295, 283,	Vitexin *p* hydroxy benzoate
30	5.253	877.2175	$C_{30}H_{40}O_{20}$	1.2	859, 757, 739, 455, 379, 325	Jatrophenol I/II/III
32	5.942	877.219	$C_{30}H_{40}O_{20}$	0.5	859, 445, 427, 409,379,349,325	Jatrophenol I/II/III
33	6.06	639.1772	$C_{32}H_{30}O_{14}$	2.1	415, 271,207	Apigenin sinapoyl hexose
34	6.13	609.159576	$C_{31}H_{28}O_{13}$	2.4	489, 433, 397, 313, 283, 177	Apigenin ferulate-*C*-hexoside
35	6.215	736.2208	$C_{33}H_{36}NO_{15}$	3.8	621,585,427,391,325, 295,177	Methylisovitexin proline ferulate ester
58	11.1	225.091	$C_{15}H_{12}O_2$	0.0	197, 165, 105	Flavanone
			Phenolic Acid Conjugates			
21	3.98	417.1347	$C_{18}H_{24}O_{11}$	−4.4	237,109	Cinnamic glycerol hexoside
23	4.06	503.1766	$C_{22}H_{30}O_{13}$	1.4	485, 467, 383	Methyl-O-feruloylquinate diacetate
52	9.9	261.1852	$C_{17}H_{24}O_2$	1.1	243, 201, 147, 119	Octyl cinnamic acid
56	10.57	221.1537	$C_{14}H_{20}O_2$	5.0	203, 161, 151, 133, 123, 105	4-Heptylbenzoic acid
74	13	291.1958	$C_{18}H_{26}O_3$	1.1	193, 123	Octyl-4-methoxycinnamate
76	13.1	235.17	$C_{15}H_{22}O_2$	3.2	179,123,57	Octyl benzoate
106	19.5	401.3422	$C_{27}H_{44}O_2$	2.0	191, 177, 137	Eicosenoyl benzoate
118	21.4	391.2837	$C_{24}H_{38}O_4$	1.2	167,149	Tetradecyl ferulate
128	22.17	419.3153	$C_{26}H_{42}O_4$	0.7	275, 293, 275,177, 127	Hexadecyl ferulate
129	23.4	463.3793	$C_{29}H_{50}O_4$	2.4	445, 417, 177,139	Nonadecyl ferulate
			Coumarins			
12	2.9	193.0492	$C_{10}H_{18}O_4$		137, 77,53	Scopoletin
15	3.31	223.0595	$C_{11}H_{10}O_5$	2.7	149, 207, 121	Fraxidin
28	5.081	501.159	$C_{22}H_{28}O_{13}$	2.5	339, 321, 177, 209	Methylhydroxycoumarin dihexoside
42	9.2	223.0738	$C_{15}H_{10}O_2$	5.7	177, 149, 121	4-Phenyl coumarin
			Nitrogenous Compounds			
1	0.465	266.1604	$C_{11}H_{23}NO_6$	2.2	248, 230, 116,104, 87	Choline hexoside
4	0.515	104.106585	$C_5H_{13}NO$	0.9	56.04, 58.06, 59.07, 60.07, 71	Choline
5	0.515	116.070497	$C_5H_9NO_2$	0.9	70	L-Proline
6	0.541	144.100864	$C_7H_{13}NO_2$	0	58, 84	Stachydrine
7	0.6	138.0549	$C_7H_7NO_2$	0.4	136, 94,92,79,66	Amino benzoic acid
8	0.65	213.1241	$C_{10}H_{16}N_2O_3$	2.3	195, 177,135,121	Prolylproline
9	0.975	146.060114	C_9H_7NO	1.0	51, 65, 77, 91, 117, 118, 128	Indole carboxyaldehyde
73	12.96	270.3153	$C_{18}H_{39}N$	0.8	158	Octadecylamine

Table 3. *Cont.*

Peak No.	tR (min)	Experimental *m/z* [M + H]$^+$	Molecular Formula	Error (ppm)	Fragments (*m/z*)	Tentative Identification
			Cyclic Peptides			
43	9.51	722.4826	$C_{36}H_{63}N_7O_8$	1.3	623, 510, 379, 280, 183	Cyclo (Val-leu-Leu-Val-Ser-Leu-Pro)
45	9.59	809.5482	$C_{40}H_{72}N_8O_9$	−1.8	722, 623, 605, 510, 280, 211	Cyclo (ABA-Val-leu-Leu-Val-Ser-Leu-Val)
46	9.6	767.5383	$C_{38}H_{70}N_8O_8$	−1.5	722, 704, 623, 510, 379	Cyclo (MeLys-leu-Leu-Val-Ser-Leu-Val)
47	9.7	652.4030	$C_{31}H_{53}N_7O_8$	0.24	634,521, 381, 268, 181	Integerrimacyclopeptide B
49	9.84	782.4596	$C_{39}H_{59}N_9O_8$	4.8	669, 651, 355, 284	Cyclogossine A
53	10.05	781.4595	$C_{40}H_{60}N_8O_8$	1.5	763, 668, 650, 555, 468	Integrimide A
54	10.53	767.5031	$C_{37}H_{66}N_8O_9$	0.7	749, 654, 636, 523, 394	Integerrimacyclopeptide A
55	10.55	668.430	$C_{32}H_{57}N_7O_8$	−3.2	650, 555, 537, 424,367,284,171	Cyclo (Leu-Leu-Gly-Thr-Leu-Ala-Val)
			Diterpenes			
31	5.814	317.2112	$C_{20}H_{28}O_3$	0.6	299, 271, 231, 175, 173	Cleistanthol/ Jatrointelone C
37	6.88	291.1951	$C_{18}H_{26}O_3$	1.3	255, 167	Triihydroxy-13-methylpodocarpane-triene
41	9.11	317.2110	$C_{20}H_{28}O_3$	−0.06	299, 271,231,175	Jatrointelone C/ Cleistanthol
51	9.9	275.2008	$C_{18}H_{26}O_2$	0.88	257,239, 173,159,119	Dihydroxy-13-methylpodocarpane-triene
59	11.36	301.21539	$C_{20}H_{28}O_2$	2.7	283 239 227 218 185	Spruceanol
61	11.95	275.2016	$C_{18}H_{26}O_2$	3.8	257, 173, 131, 91	Dihydroxy methylpodocarpane-8,10,13-triene isomer
63	11.98	315.1936	$C_{20}H_{26}O_3$	5.9	231, 199, 133,123,81	Multifidone
67	12.4	411.2355	$C_{22}H_{34}O_7$	5.4	351, 333, 369	Excolabdone C/ Isoforskolin
68	12.6	289.179	$C_{18}H_{24}O_3$	−2.8	221, 205	Methyl podocarpate
70	12.7	671.3041	$C_{36}H_{46}O_{12}$	−3.1	621, 593, 331	Premyrsinol propanoate-benzoate-triacetate
90	15.8	321.2428	$C_{20}H_{32}O_3$	1.2	275, 257	Jatrodagricaine A
94	17.57	609.2703	$C_{34}H_{40}O_{10}$	0.5	591, 531, 515, 273, 123	Diterpene benzoate triacetate
96	18	415.2391	$C_{25}H_{34}O_5$	1.8	369, 313, 295	Deoxyingenol angelate
101	18.18	651.2815	$C_{36}H_{42}O_{11}$	1.5	633, 601, 573, 483, 283	Diterpene benzoate tetracetate
103	18.65	593.2741	$C_{34}H_{40}O_9$	−0.7	547, 533, 461, 447	Diterpene benzoate triacetate
104	18.85	575.3942	$C_{34}H_{54}O_7$	0.1	309, 177	Phorbol-12-Myristate
110	20.15	695.307	$C_{38}H_{46}O_{12}$	1.1	649, 563, 517	Diterpene benzoate pentacetate
112	20.37	653.2981	$C_{36}H_{44}O_{11}$	2.4	609, 575,565, 549,503, 521	Euphorbiaproliferin F or isomer
113	20.4	637.3002	$C_{36}H_{44}O_{10}$	−1.6	619, 587, 559	Peditithin H or isomer
114	20.52	637.3018	$C_{36}H_{44}O_{10}$	0.8	619, 587,559, 473	Peditithin H or isomer
117	21.1	621.3045	$C_{36}H_{44}O_9$	−1.8	561, 533,461,433, 193	Diterpene benzoate triacetate
119	21.46	431.2432	$C_{25}H_{34}O_6$	0.9	231,165	Ingenol mebutate
123	21.9	665.3349	$C_{38}H_{48}O_{10}$	4.3	619, 587, 559	Diterpene dibenzoate diacetate
126	22.03	681.3265	$C_{38}H_{48}O_{11}$	−0.6	635, 593, 549, 503	Diterpene dibenzoate diacetate
			Sesquiterpenoids			
57	10.9	219.1751	$C_{15}H_{22}O$	3.0	203	Unidentified sesquiterpenoid
60	11.6	253.179	$C_{15}H_{24}O_3$	3.2	197, 179, 141, 151	Ilicic Acid
75	13.06	235.1700	$C_{15}H_{22}O_2$	3.2	179, 123,57	4-Patchoulen-15-oic acid
122	21.65	235.1697	$C_{15}H_{22}O_2$	1.9	179, 81	Oxo-hydroxyguai-diene
			Triterpenoids			
107	20.03	441.3736	$C_{30}H_{48}O_2$	2	423 287 235 189 149	Oxoamyrin
116	20.9	439.3573	$C_{30}H_{46}O_2$	0.6	301, 233, 173, 149, 121	Dioxo-olean-12-ene
			Fatty Acids and Their Conjugates			
38	7.46	293.2125	$C_{18}H_{28}O_3$	4.7	275, 257, 213, 195, 155	Oxo-phytodienoic acid
44	9.53	767.5324	$C_{43}H_{74}O_{11}$	2.6	623, 511	GlcADG (34:3)
62	11.95	293.2113	$C_{18}H_{28}O_3$	0.6	275, 257, 147, 133	Oxo-phytodienoic acid
64	12.26	319.2256	$C_{20}H_{30}O_3$	−5.3	301	Oxo-eicosatetraenoic acid
65	12.4	313.2362	$C_{18}H_{32}O_4$	3.6	295,277,151,95,81	Hydroperoxy-octadecadienoic acid
66	12.4	295.2278	$C_{18}H_{30}O_3$	3.5	277,179,149,151, 137, 119	Oxo-octadecadienoic acid

Table 3. *Cont.*

Peak No.	tR (min)	Experimental *m/z* [M + H]$^+$	Molecular Formula	Error (ppm)	Fragments (*m/z*)	Tentative Identification
69	12.63	277.2166	$C_{18}H_{28}O_2$	1.4	259, 135,149, 121, 107	Octadecatetraenoic acid
71	12.85	295.2276	$C_{18}H_{30}O_3$	2.8	277, 259, 231, 165	Hydroxy-Octadecatrienoic
72	12.86	351.2539	$C_{21}H_{34}O_4$	2.9	277, 259, 179, 149,133	MG 18:4
77	13.48	520.3395	$C_{26}H_{50}NO_7P$	−0.9	502,184,104	LPC 18:2
78	13.5	295.2278	$C_{18}H_{30}O_3$	6.2	277,179,149,151, 137,119	Hydroxy linoleinc acid
79	13.53	279.232	$C_{18}H_{30}O_2$	0.5	149, 95, 81	Linolenic acid
80	13.7	465.2623	$C_{22}H_{41}O_8P$	1.22	447,311,237, 155	PA: (10:1/ 9:0)
81	13.76	313.2734	$C_{19}H_{36}O_3$	1.0	295, 277, 165, 123, 95	Oxononadecanoic acid
82	13.8	331.2899	$C_{19}H_{38}O_4$	1.9	313, 257, 239, 123	2-Monopalmitin MG 16:0
83	13.94	295.2278	$C_{18}H_{30}O_3$	3.5	277,179,149,151, 137, 119	Hydroxy-octadecatrienoic acid
84	14.08	467.2784	$C_{22}H_{44}O_8$ P	2.17	393,313,239,155	PA (10:0/ 9:0)
85	14.1	295.2265	$C_{18}H_{30}O_3$	0.9	277, 179, 151	Hydroxylinolenic acid
86	14.18	496.3403	$C_{24}H_{50}NO_7P$	−0.34	478,184,125, 104, 86	LPC 16:0
87	14.3	522.3580	$C_{26}H_{52}NO_7P$	3.89	504,184,150, 104	LPC 18:1
88	15.3	291.1945	$C_{18}H_{26}O_3$	3.3	273 249 203 147	Oxo-octadecatetraenoic acid
89	15.37	723.5084	$C_{41}H_{70}O_{10}$	5.2	177, 133,89	MGDG 16:2, 16:2
91	16.095	524.3709	$C_{26}H_{54}NO_7P$	−1.36	506,184,104	LPC 18:0
92	16.19	305.2483	$C_{20}H_{32}O_2$	2.6	259, 149, 135	Arachidonic acid
93	17.1	307.2631	$C_{20}H_{34}O_2$	0.4	329, 307	Eicosatrienoic acid
95	17.64	323.2590	$C_{20}H_{34}O_3$	2.0	305, 277, 179,151	Hydroxylinoleinic acic ethyl ester
97	18.06	331.2846	$C_{19}H_{38}O_4$	0.9	313, 257, 239	1-Monopalmitin MG 16:0
98	18.07	699.5008	$C_{39}H_{70}O_{10}$	4.6	625, 607, 429	MGDG 16:2, 14:0
99	18.07	353.2665	$C_{21}H_{36}O_4$	4.0		2-Monolinolenin MG 18:3
100	18.08	745.483	$C_{43}H_{68}O_{10}$	−5.5	699, 625, 415, 295	MGDG 16:3, 18:4
102	18.24	782.5670	$C_{44}H_{80}NO_8P$	−3.8	765,307	Phosphatidylcholine (18:2/18:2)
108	20.1	359.3145	$C_{21}H_{42}O_4$	3.0	341, 285, 267, 123	Monosteirin
109	20.13	755.5713	$C_{43}H_{78}O_{10}$	3.9	681,663,443,323	MGDG 18:2/16:0
111	20.15	381.2996	$C_{23}H_{40}O_4$	0.9	none	MG 20:3
115	20.89	549.4527	$C_{34}H_{60}O_5$	2.5	531, 513, 287, 189, 121	DG 14:1/17:2
121	21.6	313.2723	$C_{19}H_{36}O_3$	4.5	295, 277, 149, 133	Ricinoleate methyl esters
125	22	613.4828	$C_{39}H_{64}O_5$	0.2	595, 335, 261	Dilinolenin DG 18:3/18:3
132	25.1	591.4920	$C_{37}H_{66}O_5$	−6.3	573, 335,313,261	DG 18:3/16:0
			Phytosterols			
105	19.36	445.3630	$C_{29}H_{48}O_3$	−4.6	427, 341, 185	Oxo-hydroxy sitosterol
120	21.55	413.3792	$C_{29}H_{48}O$	2.07	395, 159	Stigmasterol
124	21.9	429.3723	$C_{29}H_{48}O_2$	0.9	411	Stigmast-4-en-6beta-ol-3-one
127	22.15	461.3616	$C_{29}H_{48}O_4$	2.0	443, 401, 383, 187	Trihydroxystigmastan-6-one ene
130	23.5	415.3964.8	$C_{29}H_{50}O$	4.8	397, 341	Sitosterol
131	23.7	429.3729	$C_{29}H_{48}O_2$	0.2	219, 205, 165	Stigmastane 3,6 dione
			Jasmonates			
36	6.263	265.1433	$C_{15}H_{20}O_4$	0.5	247, 219,207,205,167,99 191	Abscisic acid
39	7.55	181.122163	$C_{11}H_{16}O_2$	9.4	163,135, 121, 107, 99, 93	Jasmorolone
40	8.6	255.1498	$C_{13}H_{20}O_3$	5.7	195, 179, 143,137	Methyljasmonate
			Miscellaneous Compounds			
2	0.47	365.1069	$C_{14}H_{20}O_{11}$	2.6	203, 185	Ethyl aconitate hexoside
3	0.48	527.1584	$C_{20}H_{30}O_{16}$	4.8	365, 347, 203, 185	Ethyl aconitate dihexoside
17	3.56	197.1168	$C_{11}H_{16}O_3$	−2.1	179,161,135, 107	Loliolide/Epiloliolide
18	3.63	197.1170	$C_{11}H_{16}O_3$	−1.2	179, 161, 133, 105, 91	Loliolide/Epiloliolide
48	9.8	409.166	$C_{24}H_{24}O_6$	3.5	289, 121, 119	Benzyl shikonin
50	9.88	387.1793	$C_{22}H_{26}O_6$	−2.3	267, 147, 121, 105	Eudesmin/epieudesmin
133	25.45	417.3652	$C_{28}H_{48}O_2$	0.3	191, 151	Tocopherol

DG: diglyceride; LPC: lysophosphatidylcholine; MG: monoglyceride; MGDG: monoglyceridedi galactoside; PA: phosphatidic acid.

2.4.2. Nitrogenous Compounds

Eight simple nitrogenous compounds were annotated in *J. integerrima* extract, including choline and its glycoside (peaks 4 and 1, respectively). Other simple nitrogenous compounds included proline (peak 5) and its betaine derivative stachydrine (peak 6) in addition to proylproline (peak 8).

Apart from the simple nitrogenous compounds, 8 cyclic peptides (hepta-and octa-peptides) were observed between retention time of 9.5–10.5 min. Five cyclic peptides were

previously isolated from the latex of *J. integerrima* [12,26,27] including integerrimide A (peak 53, $[M + H]^+$ at m/z 781.4595 calculated for $C_{40}H_{61}N_8O_8$) [12], integrimmacyclopeptide A (peak 54, $[M + H]^+$ at m/z 767.5031 calculated for $C_{37}H_{67}N_8O_9$), and integrrimacyclopeptide B (peak 47, $[M + H]^+$ at m/z 652.4030 calculated for $C_{31}H_{54}N_7O_8$). Additionally, four cyclic peptides, which were not previously reported, were annotated in peaks 43, 45, 46, and 55, Figure 6. The amino acid sequence of these peptides was tentatively determined based on the high-resolution mass of their fragment ions Table S1, Figures S8–S11. These new cyclic peptides consisted mainly of hydrophobic residues including leucine or isoleucine (neutral loss of 113 Da, corresponding to $C_6H_{11}NO$) and valine (neutral loss of −99 Da corresponding to C_5H_9NO), Table S1, Figure 6.

2.4.3. Terpenoids

The most abundant group of secondary metabolites detected in *J. integerrima* leaves was that of terpenoids including sesquiterpenes (4 metabolites), diterpenes (24 metabolites) and two triterpenoids. To date, only free diterpenoids or their monoacetyl derivatives were reported in *J. integerrima* [14,15,28]. In this study, free diterpenoids were eluted first at retention time 5–11 min and were of cleistanthane, lathyrane, and podocarpane scaffold containing two or three oxygen atoms (peaks 31, 37, 41, 51, 59, 61, 63, and 68, Table 3, Figures 6 and S12–S14).

Meanwhile, diterpenoids esters were eluted at retention time 12–22 min and were mostly esters of jatrophane, phorbol, and ingenol. Acetate esters were predominant and were readily identified by the neutral loss of acetic acid (−60 Da) as seen in peaks 67, 70, 94, 112, 113, 114, and 117, while the presence of angelate ester (peak 96) was inferred from the neutral loss of 102 Da. Additionally esterification with benzoic acid was observed in late eluting peaks as indicated by the increase in double bond equivalent (14–16). These were observed in peaks 103 ($C_{34}H_{40}O_9$), 110 ($C_{38}H_{46}O_{12}$), 113,114 ($C_{36}H_{44}O_{10}$), 117 ($C_{36}H_{44}O_9$), 123 ($C_{38}H_{48}O_{10}$), and 126 ($C_{38}H_{48}O_{11}$) which showed multiple loss of acetate (−60 Da) and fragment ion at *m/z* 123 for benzoic acid, Figures S15 and S16. This substitution pattern of acetate and benzoate esters of a highly oxygenated skeleton has never reported before in *J. integerrima*. However, among Euphorbiaceae plants, molecules with highly oxygenated jatrophane, esterified with benzoic and acetic acid, were isolated from *Pedilanthus tithymaloides* and are designated as pedititihin [29], and from *Euphobia sanctae-catharinae* are designated as euphosantiananes, [30,31]. Due to lack of extensive fragmentation of these high molecular weight esters, the exact carbon skeleton of these diterpenes could not be identified.

Among sesquiterpenes annotated in this study, an oxo-hydroxyguai-diene derivative was annotated ($C_{15}H_{22}O_2$, peak 122), which was previously isolated from *J. integerrima* [11]. Two triterpenoids were annotated, namely, oxoamyrin and dioxo-olean-12-ene.

2.4.4. Fatty Acids and Their Conjugates

Fatty acids were detected as free fatty acid, fatty acid glycerides, phospholipids, and glycolipids, which together accounted for 38 metabolites. Unsaturated fatty acids C-18 were most abundant including oxo-phytodienoic ($C_{18}H_{28}O_3$), linolenic ($C_{18}H_{30}O_2$), hydroxylinoleinic ($C_{18}H_{30}O_3$), oxo-octadecadienoic ($C_{18}H_{30}O_3$) and ricinoleic acids. Several fatty acids derivatives were annotated in the extract including monoglyceride (MG), diglycerides (DG), monogalactosyl diglycerides (MGDG), phosphatidic acid esters (PA), and lysophasphatidyl choline (LPC).

Monoglycerides showed two consecutive loss of water (−18 Da), in addition to loss of −92 Da corresponding to loss of glycerol moiety (peaks 82, 108, and 111, Figure S17). Meanwhile, phosphatidic acid esters displayed a characteristic loss of 154 Da and appearance of a fragment ion at 155 amu corresponding to phosphopropionic acid ($C_3H_7O_5P$) as seen in peaks 80 and 84, Table 3, Figure S18. Annotated phosphatidic acid esters were diglyceride with medium chain length (C-9 and C-10) and were observed in peaks 80 and 84. Fatty acid esters containing choline showed characteristic choline fragment at *m/z* 104.106 as in peaks 86, 87, and 91, Figure S19. Additionally, monogalactosyl diglycerides MGDG

were identified in peaks 89, 98, 100, and 109, mainly as monoglycerides of C-16 or C-18 fatty acids.

2.4.5. Phytosterols and Other Compounds

Six phytosterols were annotated in the UPLC/MS chromatogram of *J. integerrima* including β-sitosterol, stigmasterol, stigmastane 3,6 dione and stigmast-4-en-6beta-ol-3-one, which all were previously reported in the genus *Jatropha* and the latter was isolated from *J. integerrima*. [11]. Three jasmonates (peaks 36, 39, and 40), two terpene lactones (peaks 17, 18), and tocopherol (peak 133) were also among annotated compounds.

2.5. Quantitative Determination of Secondary Metabolites

To gain possible insights into the relative abundance of secondary metabolites in *J. integerrima* extract, spectrophotometric methods were used to estimate the abundance of certain classes of secondary metabolites. Leaves' extract of *J. integerrima* showed low abundance of phenolic compounds and flavonoids at 70.4 ± 0.4 mg GAE and 10.7 ± 0.1 mg QE per gram of extract, respectively. Meanwhile, results from vanillin/sulfuric assay indicated a high terpenoidal content of 149.7 mg UAE per gram of the extract.

3. Discussion

Leaves of *Jatropha integerrima* were used in South-East Asia (particularly in India and Bangladesh) for treatment of some inflammatory conditions such as arthritis and eczema. Other *Jatropha* species have well documented anti-inflammatory activity as proven by folk use, in vitro and in vivo assays [4,32,33]. Our investigation showed that *Jatropha integerrima* leaves extract (JILE) possess an anti-inflammatory effect when used in rat paw edema model. Both oral administration and topical application of the extract were able to reduce signs of inflammation (edema volume) and levels of inflammatory mediators (NO, TNF-α, PKC, and PGE2). Inflammation induced by carrageenan increased paw edema thickness and was associated with elevated levels of NO, TNF-α, PKC, and PGE2 after 4 h, as previously shown in other studies [34]. The involvement of NO and PGs in the modulation of inflammation is well established [35]. Lipid peroxidation initiated by the product of the reaction of NO with superoxide (peroxynitrite) liberates arachidonic acid from the cell membrane activating PGE2, which is one of the strongest inflammatory mediators [36]. Moreover, TNF-α induces NO synthesis by activating inducible nitric oxide synthase iNOS and augments the responses of neutrophils to inflammatory stimuli [37,38]. Activation of PKC mediates the activation of NF-κB, and secretion of TNF-α, IL-6, and IL-10 through TLR2/1 [39]; this may explain the elevation of PKC that provoked the increase in TNF-α serum level in our work.

Treatment with JILE decreased levels of these inflammatory mediators in a dose dependent manner and restored their basal levels at higher treatment dose of 400 mg/kg (per oral route) and using 10% strength cream (after topical application). Additionally, normal tissue structure was observed in animals receiving JILE treatment especially with topical application of 5% or 10% cream or using an oral dose of 400 mg/kg confirming the ability of JILE extract to inhibit inflammatory response.

The metabolomics profile of *J. integerrima* identified many compounds with known anti-inflammatory activity. For example, 19 flavonoidal compounds were detected in the extract which were mainly apigenin derivatives. Apigenin and its C-glycosides (vitexin and isovitexin) are known anti-inflammatory compounds that protect cells against oxidative stress and inflammation [40–42]. The major flavonodial compound in the extract (vitexin) was shown to reduce the levels of inflammatory cytokines such as IL4, IL5, and IL13 by 64%, 95%, and 65% in an induced asthma model at concentration of 1 mg/kg [43].

In addition to flavonoids, coumarins such as scopoletin and fraxidin have anti-inflammatory action mediated by their antioxidant potential [44–46]. Scopoletin can reduce inflammation and level of PGE2 in LPS stimulated cell lines [47].

Finally, nonpolar compounds such as triterpenes, sterols, and diterpenes are usually main contributors to anti-inflammatory activity of plant extracts. As reported previously, the nonpolar *n*-hexane fraction of *Jatropha curcas* extract had a better anti-inflammatory activity when tested in vitro compared to the polar fraction [33]. Additionally, *J.integerrima* extract was enriched with oxo and hydroxyl fatty acids (13 compounds, Table 3), which received attention recently due to their potential as anti-inflammatory agents and their indigenous role in regulating body inflammatory response [48].

4. Conclusions

These results suggest that the ethanol extract of the leaves of *Jatropha integerrima* possess a significant anti-inflammatory effect, both through oral administration and topical application. This effect can be attributed to its high content of flavonoids, terpenoids, and oxygenated fatty acids, which are represented in the metabolomics profile of the extract by 65 compounds. Our findings encourage further investigation of the rich metabolome of *J. integerrima* to identify novel anti-inflammatory compounds.

5. Material and Methods

5.1. Plant Material

Fresh leaves of *Jatropha integerrima* Jacq. were collected from plants cultivated at the Medicinal, Aromatic and Poisonous Plants Experimental Station of the Faculty of Pharmacy, Cairo University (Giza, Egypt). Plant identity was confirmed by Therese Labib, consultant of plant taxonomy at the Ministry of Agriculture and Orman Botanical Garden (Giza, Egypt). Vouchered herbarium specimen (code numbers 582018III) was deposited at the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

5.2. Preparation of Plant Extract

Fresh leaves were air dried in shade and reduced to powder. The powdered leaves (1 kg) were extracted with absolute ethanol by cold maceration till exhaustion (4 × 5 L). The solvent was then removed by vacuum distillation at a temperature not exceeding 40 °C, and the dried ethanol extract was kept in an air-tight container at 4 °C till use.

5.3. Determination of Total Phenolic Content

Total phenolic content of *J. integerrima* leaves extract was carried out using the Folin–Ciocalteu method following the optimized assay procedures described by Blainski, Lopes, and de Mello [49]. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g of extract using a standard calibration curve of gallic acid (20–200 µg/mL).

5.4. Determination of Flavonoid Content

Total flavonoid content was determined by measuring the yellow color developed upon reacting flavonoids with $AlCl_3$ reagent according to optimized assay conditions described by Silva et al. [50]. The total flavonoid content was expressed in mg quercetin equivalent (QE)/g of extract based on pre-established calibration curve of quercetin (0.1–0.7 mg/mL).

5.5. Determination of Steroidal/Terpenoidal Content

Spectrophotometric estimation of total steroidal and/or terpenoid content was carried out based on the chromogenic reaction produced upon treatment of the extract with vanillin/ sulfuric acid reagent according to the protocol developed by V. Le et al. [51]. The color developed was measured at λ = 544 nm, and total steroid and/or terpenoid content was expressed as ursolic acid equivalent (UAE mg/g of extract) as deduced from a pre-established calibration curve using standard ursolic acid (20–160 µg/mL).

5.6. Ultra-Performance Liquid Chromatography Analysis

Liquid chromatography instrument (Sciex, TripleTOF 5600+, Framingham, MA, USA) was used for chromatographic separation. A reversed phase C18 analytical column (Waters, Xbridge C18, 2.1 × 50 mm, 3.5 µm particle size) was used at 40 °C. Leaves extract (50 mg) was dissolved in 1 mL of water: methanol: acetonitrile (50:25:25) and centrifuged for 5 min at 10,000 rpm followed by filtration through a membrane disc filter. Twenty microliters were diluted to 1 mL and 10 µL of this solution was injected into the system. Gradient elution was carried out at a flow rate of 0.3 mL/min using eluent A (0.1% formic acid in deionized water) and eluent B (100% acetonitrile). Elution was performed according to the following gradient: 10% B, 0–1 min; 10%–90% B, 1–25 min; 90%–10% B, 25–28 min.

5.7. High Resolution Quadrpole-Time of Flight Mass Analysis

UPLC system was coupled to electrospray ion source with quaderpole-time of flight mass analyzer (ESI-QTOF, Framingham, MA, USA). MS analysis was performed in positive ion mode; cone voltage, 30 eV; capillary voltage, 3 kV; desolvation temperature, 450 °C; cone gas flow, 50 L/h and desolvation gas flow, 900 L/h. Time of flight mass scan (TOF-MS, Framingham, MA, USA) was controlled using Analyst TF 1.7.1 and was recorded over *m/z* range 50–1000. TOF MS/MS scan was done in information dependent acquisition over the same mass range. UPLC-MS/MS data were processed with PeakView 2.2 (Framingham, MA, USA) for data extraction.

5.8. Anti-Inflammatory Assay

5.8.1. Animals

Adult male Wister albino rats (7–8 weeks old, weighing 130–180 g) were housed in standard cages (10 rats/cage) under pathogen-free conditions and maintained at controlled room temperature (21–24 °C) with a 40–60% relative humidity and under normal dark-light cycles. All animals had free access to rat chew diet and water *ad libitum*. All procedures were approved by the Animal Care Committee of the National Research Centre.

5.8.2. Acute Toxicity Study

Rats were divided into two groups of 12 rats each (6 males and 6 females). The extract of *J. integerrima* was suspended in distilled water and given orally to rats of the first group in a single dose of 5 g/kg. The control group received the same volumes of distilled water. The percentage mortality for the extract was recorded for 24 h. Animals were observed for 14 days, for any changes in skin, fur, respiratory, circulatory, central nervous system, somatomotor activity, and behavior pattern. Particular observation for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma were also recorded.

5.8.3. Carrageenan Induced Paw Edema

Anti-inflammatory activity was evaluated using carrageenan induced paw edema. Paw swelling was elicited by subplantar injection of 100 µL of 1% sterile lambda carrageenan suspension in saline into the right hind paw [35]. Contralateral paw received an equal volume of saline solution. The edema component of inflammation was quantified by measuring hind footpad immediately before carrageenan injection and 1–4 h after carrageenan injection with a micrometer caliber [52]. Edema was expressed as a percentage of change from control (predrug) values. Rats, to be injected with carrageenan, were divided into nine groups each of 10 animals. Group 1 (control group): where rats received oral saline (0.2 mL/rat); group 2: rats were given indomethacin (25 mg/kg); groups 3 and 4: rats received the extract of *J. integerrima* (200 mg/kg and 400 mg/kg), respectively. Indomethacin and the extracts were given orally 60 min before the injection of the carrageenan suspension.

5.8.4. Skin Irritation Test

Twelve rats were divided into two groups (six rats each), namely, control and test groups. Hairs of rat hind paw were shaved, and rats of different groups were kept in separate cages for seven days. An amount (0.3 g) of 10% cream was placed on the shaved skin (4 cm^2) for rats of the test group while the other group received only base cream. The area was covered with a cotton bandage and any sensitivities were assessed and graded.

5.8.5. Topical Anti-Inflammatory Activity

The base cream was prepared according to recipe by Franyoto et al.(2018) [53] containing stearic acid (142 g), glycerine (100 g), sodium tetraborate (2.5 g), triethanolamine (10 g), methyl paraben (0.1 g) and 750 mL of distilled water. The base cream was then mixed with the ethanol extract of the leaves of *Jatropha integerrima* at three different concentrations (2.5%, 5%, and 10%) [53].

Before edema induction as described in Section 5.8.3, rats were divided into six groups each of 10 rats. Group 1 was left untreated, while animals in group 2 were given a single topical dose (0.3 g) of market product of hydrocortisone (1%). Group 3 rats received a single topical dose (0.3 g) of base cream, while animals in groups 4, 5, and 6 received 0.3 g of the cream extract of *J. integerrima* at concentrations of 2.5%, 5%, and 10%, respectively. An amount of 0.3 g of the tested cream was used 30 min before injection of the carrageenan suspension, and it was gently rubbed 50 times with the index finger. A separate group of healthy animals were kept as a control group. Edema was expressed as a percentage change from control (predrug) values [54].

5.8.6. Blood Samples and Biochemical Analysis

Four hours after carrageenan injection and immediately after measuring edema volume, animals were anesthetized with urethane (1.5 g/kg; i.p.) and blood samples were taken from the abdominal aorta and used for determination of PGE2 using Abnova ELISA Kit, TNF-α using Cusabio ELISA Kit, and PKC by enzyme linked immunoassay (ELISA) technique using standard kits (Glory Science Co., Ltd, Louisiana, LA, USA).

5.8.7. Histological Examination

Paws were fixed in 10% formalin solution and dehydrated in ascending grades of alcohol and embedded in paraffin. Sections at four-micron thickness were taken and stained with hematoxylin and eosin (H&E).

5.9. Statistical Analysis

All the values are presented as mean ± standard error of the means (SE). Comparisons between different groups were carried out using one-way analysis of variance (ANOVA), followed by Tukey's HSD test for multiple comparisons. Graph pad Prism software, version 5 (GraphPad Software Inc., San Diego, CA, USA), was used to carry out statistical tests. The difference was considered significant when $p < 0.05$.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants11020218/s1, Figure S1: MS/MS spectrum of peak 29: Vitexin-*p*-hydroxybenzoate; Figure S2: Fragmentation pattern highlighting different fragmentation of the two structural isomer vitexin and isovitaxin based on the intensity of 313 and 283 fragment ions; Figure S3: MS/MS spectrum of peaks 30 and 32: Jatrophenol I/II/III; Figure S4: MS/MS spectrum of peak 35: Proline methylisovitexin; Figure S5: MS/MS spectrum of peak 35: Methylisovitexin proline ferulate; Figure S6: MS/MS spectrum of peak 26: Apigenin-*O*-hexoside; Figure S7: MS/MS spectrum of peak 25: Apigenin-*O*-hexoside-*O*-rhamnoside; Figure S8: MS/MS spectrum of peak 43: A cycloheptapeptide; Figure S9: MS/MS spectrum of peak 45: A cyclooctapeptide; Figure S10: MS/MS spectrum of peak 46: A cycloheptapeptide; Figure S11: MS/MS spectrum of peak 46: A cycloheptapeptide; Figure S12: MS/MS spectrum of peak 59: Spruceanol; Figure S13: MS/MS spectrum of peak 61; Figure S14: MS/MS for peak 67: Isoforskolin; Figure S15: MS/MS for peak 114: Premyrsinol/peditithin derivatives; Figure S16: MS/MS of peak 125: Premyrsinol/peditithin derivative; Figure S17: MS/MS of peak 82:

Monopalmitin; Figure S18: MS/MS of peak 84: Phosphatidic acid (10:0/9:0); Figure S19: MS/MS of peak 86: Lysophosphatidylcholine (16:0/0:0); Table S1: Analysis of high resolution MS/MS Q-TOF fragments for newly identified cyclic peptides.

Author Contributions: Conceptualization: S.M.E.-Z. and A.M.S.; methodology, A.H.E.; software, E.A.M.; validation, A.A.A.S. and A.H.E.; formal analysis, A.H.E. and E.A.M.; investigation, A.H.E., E.A.M. and A.A.A.S.; resources, S.M.E.-Z. and A.M.S.; data curation, E.A.M.; writing—original draft preparation, E.A.M. and A.A.A.S.; writing—review & editing, A.H.E. and S.M.E.-Z.; supervision, A.M.S. and S.M.E.-Z. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: All data related to this article are presented in this manuscript or available as supplementary material.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cavalcante, N.B.; Diego da Conceição Santos, A.; Guedes da Silva Almeida, J.R. The genus *Jatropha* (Euphorbiaceae): A review on secondary chemical metabolites and biological aspects. *Chem. Biol. Interact.* **2020**, *318*, 108976. [CrossRef]
2. Srinivasan, N.; Palanisamy, K.; Mulpuri, S. Jatropha: Phytochemistry, Pharmacology, and Toxicology. In *Jatropha, Challenges for a New Energy Crop*; Mulpuri, S., Carels, N., Bahadur, B., Eds.; Springer: Singapore, 2019; pp. 415–435.
3. Sabandar, C.W.; Ahmat, N.; Jaafar, F.M.; Sahidin, I. Medicinal property, phytochemistry and pharmacology of several *Jatropha* species (Euphorbiaceae): A review. *Phytochemistry* **2013**, *85*, 7–29. [CrossRef]
4. Salim, M.N.; Masyitha, D.; Harris, A.; Balqis, U.; Iskandar, C.D.; Hambal, M. Darmawi Anti-inflammatory activity of *Jatropha curcas* Linn. latex in cream formulation on CD68 expression in mice skin wound. *Vet. World* **2018**, *11*, 99–103. [CrossRef] [PubMed]
5. Abdulkarim, B.; Al-Maqtari, T.; Al-Doaiss, A.A.; Dhabali, A.A.H. Antioxidant, antimicrobial and wound healing potential of *Jatropha variegata* -An interesting plant endemic to Yemen. *Pakistan J. Biol. Sci.* **2020**, *23*, 1581–1590. [CrossRef] [PubMed]
6. Hernandez-Hernandez, A.B.; Alarcon-Aguilar, F.J.; Garcia-Lorenzana, M.; Rodriguez-Monroy, M.A.; Canales-Martinez, M.M. *Jatropha neopauciflora* Pax latex exhibits wound-healing effect in normal and diabetic mice. *J. Evid.-Based Integr. Med.* **2021**, *26*, 2515690X2098676. [CrossRef] [PubMed]
7. Verardo, G.; Baldini, M.; Ferfuia, C.; Gorassini, A. Rapid and selective screening for toxic phorbol esters in *Jatropha curcas* seed oil using high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *J. Chromatogr. A* **2019**, *1597*, 63–75. [CrossRef]
8. Roach, J.S.; Devappa, R.K.; Makkar, H.P.S.; Becker, K. Isolation, stability and bioactivity of *Jatropha curcas* phorbol esters. *Fitoterapia* **2012**, *83*, 586–592. [CrossRef]
9. Akhter, A.; Rahman, M.S.; Ahsan, M. Preliminary antimicrobial and cytotoxic activities of n-hexane extract of *Jatropha pandurifolia*. *Lat. Am. J. Pharm.* **2008**, *27*, 918–921.
10. Wagh, V.V.; Jain, A.K. Ethnopharmacological survey of plants used by the Bhil and Bhilala ethnic community in dermatological disorders in Western Madhya Pradesh, India. *J. Herb. Med.* **2020**, *19*, 100234. [CrossRef]
11. Sutthivaiyakit, S.; Mongkolvisut, W.; Prabpai, S.; Kongsaeree, P. Diterpenes, sesquiterpenes, and a sesquiterpene−coumarin conjugate from *Jatropha integerrima*. *J. Nat. Prod.* **2009**, *72*, 2024–2027. [CrossRef]
12. Mongkolvisut, W.; Sutthivaiyakit, S.; Leutbecher, H.; Mika, S.; Klaiber, I.; Möller, W.; Rösner, H.; Beifuss, U.; Conrad, J. Integerrimides A and B, cyclic heptapeptides from the latex of *Jatropha integerrima*. *J. Nat. Prod.* **2006**, *69*, 1435–1441. [CrossRef]
13. Zhu, J.-Y.; Cheng, B.; Zheng, Y.-J.; Dong, Z.; Lin, S.-L.; Tang, G.-H.; Gu, Q.; Yin, S. Enantiomeric neolignans and sesquineolignans from *Jatropha integerrima* and their absolute configurations. *RSC Adv.* **2015**, *5*, 12202–12208. [CrossRef]
14. Zhang, D.-B.; Wang, Z.; Liang, Y.-N.; Yu, J.-G.; Zhang, Z.; Liu, S.-J.; Zhang, Z.; Song, Z.-X.; Tang, Z.-S.; Duan, D.-Z. Jatrophainolides A–C, new cembrane-type diterpenoids with PTP1B inhibitory activity from the root bark of *Jatropha integerrima*. *Phytochem. Lett.* **2020**, *36*, 166–170. [CrossRef]
15. Zhu, J.-Y.; Lou, L.-L.; Guo, Y.-Q.; Li, W.; Guo, Y.-H.; Bao, J.-M.; Tang, G.-H.; Bu, X.-Z.; Yin, S. Natural thioredoxin reductase inhibitors from *Jatropha integerrima*. *RSC Adv.* **2015**, *5*, 47235–47243. [CrossRef]
16. Mohammed, A.E.; Al-Keridis, L.A.; Rahman, I.; Alotaibi, M.O.; Suliman, R.S.; Alrajhi, A.M.; Elobeid, M.M.; Alothman, M.R.; Alhomaidi, E.A.; Korany, S.M. Silver Nanoparticles Formation by *Jatropha integerrima* and LC/MS-QTOF-Based Metabolite Profiling. *Nanomaterials* **2021**, *11*, 2400. [CrossRef] [PubMed]
17. Eshilokun, A.O.; Kasali, A.A.; Ogunwande, I.A.; Walker, T.M.; Setzer, W.N. Chemical composition and antimicrobial studies of the essential oils of *Jatropha integerrima* Jacq (leaf and seeds). *Nat. Prod. Commun.* **2007**, *2*, 853–855. [CrossRef]
18. Xu, D.-P.; Zhou, Y.; Zheng, J.; Li, S.; Li, A.-N.; Li, H.-B. Optimization of ultrasound-assisted extraction of natural antioxidants from the flower of *Jatropha integerrima* by response surface methodology. *Molecules* **2016**, *21*, 18. [CrossRef]
19. Cong, H.H.; Khaziakhmetova, V.N.; Zigashina, L.E. Rat paw oedema modeling and NSAIDs: Timing of effects. *Int. J. Risk Saf. Med.* **2015**, *27*, S76–S77. [CrossRef] [PubMed]
20. Marzouk, M.S.; Moharram, F.A.; Haggag, E.G.; El-Batran, S.M.; Mahmoud, I.I.; Ibrahim, R.R. Novel biflavone diglycosides and biological activity of *Jatropha multifida* leaves. *Chem. Nat. Compd.* **2012**, *48*, 765–770. [CrossRef]

21. Zengin, G.; Mahomoodally, M.F.; Sinan, K.I.; Ak, G.; Etienne, O.K.; Sharmeen, J.B.; Brunetti, L.; Leone, S.; Di Simone, S.C.; Recinella, L.; et al. Chemical composition and biological properties of two *Jatropha* species: Different parts and different extraction methods. *Antioxidants* **2021**, *10*, 792. [CrossRef]
22. Moharram, F.; Marzouk, M.; Haggag, E.; El-Batran, S.; Ibrahim, R. Biological examination and novel biflavone di-C-glycosides from *Jatropha multifida* L. leaves. *Planta Med.* **2007**, *73*, P048. [CrossRef]
23. Mekky, R.H.; Thabet, M.M.; Rodríguez-Pérez, C.; Elnaggar, D.M.Y.; Mahrous, E.A.; Segura-Carretero, A.; Abdel-Sattar, E. Comparative metabolite profiling and antioxidant potentials of seeds and sprouts of three Egyptian cultivars of *Vicia faba* L. *Food Res. Int.* **2020**, *136*, 109537. [CrossRef] [PubMed]
24. Ravindranath, N.; Ravinder Reddy, M.; Ramesh, C.; Ramu, R.; Prabhakar, A.; Jagadeesh, B.; Das, B. New lathyrane and podocarpane diterpenoids from *Jatropha curcas*. *Chem. Pharm. Bull.* **2004**, *52*, 608–611. [CrossRef] [PubMed]
25. Das, B.; Ravikanth, B.; Laxminarayana, K.; Ramarao, B.; Raju, T.V. New Macrocyclic diterpenoids from *Jatropha multifida*. *Chem. Pharm. Bull.* **2009**, *57*, 318–320. [CrossRef]
26. Welé, A.; Baraguèye, C.; Ndiaye, W.; Fall, D.; Ndoye, I.; Diop, Y.; Dubosq, L.; Bodo, B. [Cytotoxic activity of two cyclic peptides from the latex of *Jatropha integerrima* Euphorbiaceae]. *Dakar Med.* **2007**, *52*, 209–215. [PubMed]
27. Idrissa, N.; Adama, D.; Mamadou, B.; Rokhaya, S.; Yoro, T.; Alassane, W.; Djibril, F. Novel cytotoxic cycloheptapeptide from the Latex of *Jatropha integerrima*. *J. Chem. Pharm. Res.* **2016**, *8*, 135–139.
28. Sutthivaiyakit, S.; Mongkolvisut, W.; Ponsitipiboon, P.; Prabpai, S.; Kongsaeree, P.; Ruchirawat, S.; Mahidol, C. A novel 8,9-seco-rhamnofolane and a new rhamnofolane endoperoxide from *Jatropha integerrima* roots. *Tetrahedron Lett.* **2003**, *44*, 3637–3640. [CrossRef]
29. Zhu, J.; Wang, R.; Lou, L.; Li, W.; Tang, G.; Bu, X.; Yin, S. Jatrophane diterpenoids as modulators of P-glycoprotein-dependent multidrug resistance (MDR): Advances of structure-activity relationships and discovery of promising MDR reversal agents. *J. Med. Chem.* **2016**, *59*, 6353–6369. [CrossRef]
30. Hegazy, M.-E.; Hamed, A.; Ibrahim, M.; Talat, Z.; Reda, E.; Abdel-Azim, N.; Hammouda, F.; Nakamura, S.; Matsuda, H.; Haggag, E.; et al. Euphosantianane A–D: Antiproliferative premyrsinane diterpenoids from the endemic Egyptian plant *Euphorbia sanctae-catharinae*. *Molecules* **2018**, *23*, 2221. [CrossRef]
31. Elshamy, A.I.; Mohamed, T.A.; Al-Rowaily, S.L.; Abd-ElGawad, A.M.; Dar, B.A.; Shahat, A.A.; Hegazy, M.-E.F. Euphosantianane E–G: Three new premyrsinane type diterpenoids from *Euphorbia sanctae-catharinae* with Contribution to chemotaxonomy. *Molecules* **2019**, *24*, 2412. [CrossRef]
32. Mujumdar, A.; Misar, A. Anti-inflammatory activity of *Jatropha curcas* roots in mice and rats. *J. Ethnopharmacol.* **2004**, *90*, 11–15. [CrossRef]
33. Othman, A.R.; Abdullah, N.; Ahmad, S.; Ismail, I.S.; Zakaria, M.P. Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root. *BMC Complement. Altern. Med.* **2015**, *15*, 11. [CrossRef]
34. Abd El-Alim, S.H.; Kassem, A.A.; Basha, M.; Salama, A. Comparative study of liposomes, ethosomes and transfersomes as carriers for enhancing the transdermal delivery of diflunisal: In vitro and in vivo evaluation. *Int. J. Pharm.* **2019**, *563*, 293–303. [CrossRef]
35. Salvemini, D.; Wang, Z.-Q.; Bourdon, D.M.; Stern, M.K.; Currie, M.G.; Manning, P.T. Evidence of peroxynitrite involvement in the carrageenan-induced rat paw edema. *Eur. J. Pharmacol.* **1996**, *303*, 217–220. [CrossRef]
36. Mariotto, S.; Suzuki, Y.; Persichini, T.; Colasanti, M.; Suzuki, H.; Cantoni, O. Cross-talk between NO and arachidonic acid in inflammation. *Curr. Med. Chem.* **2007**, *14*, 1940–1944. [CrossRef]
37. Salim, T.; Sershen, C.L.; May, E.E. Investigating the role of TNF-α and IFN-γ activation on the dynamics of iNOS gene expression in LPS stimulated macrophages. *PLoS ONE* **2016**, *11*, e0153289. [CrossRef] [PubMed]
38. Saini, R.; Singh, S. Inducible nitric oxide synthase: An asset to neutrophils. *J. Leukoc. Biol.* **2019**, *105*, 49–61. [CrossRef] [PubMed]
39. Loegering, D.J.; Lennartz, M.R. Protein kinase C and toll-like receptor signaling. *Enzyme Res.* **2011**, *2011*, 1–7. [CrossRef] [PubMed]
40. He, M.; Min, J.-W.; Kong, W.-L.; He, X.-H.; Li, J.-X.; Peng, B.-W. A review on the pharmacological effects of vitexin and isovitexin. *Fitoterapia* **2016**, *115*, 74–85. [CrossRef] [PubMed]
41. Peng, Y.; Gan, R.; Li, H.; Yang, M.; McClements, D.J.; Gao, R.; Sun, Q. Absorption, metabolism, and bioactivity of vitexin: Recent advances in understanding the efficacy of an important nutraceutical. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 1049–1064. [CrossRef] [PubMed]
42. Ginwala, R.; Bhavsar, R.; Chigbu, D.I.; Jain, P.; Khan, Z.K. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants* **2019**, *8*, 35. [CrossRef] [PubMed]
43. Venturini, C.L.; Macho, A.; Arunachalam, K.; de Almeida, D.A.T.; Rosa, S.I.G.; Pavan, E.; Balogun, S.O.; Damazo, A.S.; de Oliviera Martins, D.T. Vitexin inhibits inflammation in murine ovalbumin-induced allergic asthma. *Biomed. Pharmacother.* **2018**, *97*, 143–151. [CrossRef] [PubMed]
44. Whang, W.K.; Park, H.S.; Ham, I.; Oh, M.; Namkoong, H.; Kim, H.K.; Hwang, D.W.; Hur, S.Y.; Kim, T.E.; Park, Y.G.; et al. Natural compounds, fraxin and chemicals structurally related to fraxin protect cells from oxidative stress. *Exp. Mol. Med.* **2005**, *37*, 436–446. [CrossRef]
45. Jamuna, S.; Karthika, K.; Paulsamy, S.; Thenmozhi, K.; Kathiravan, S.; Venkatesh, R. Confertin and scopoletin from leaf and root extracts of Hypochaeris radicata have anti-inflammatory and antioxidant activities. *Ind. Crops Prod.* **2015**, *70*, 221–230. [CrossRef]

46. Ding, Z.; Dai, Y.; Hao, H.; Pan, R.; Yao, X.; Wang, Z. Anti-Inflammatory effects of scopoletin and underlying mechanisms. *Pharm. Biol.* **2008**, *46*, 854–860. [CrossRef]
47. Kim, H.-J.; Jang, S.I.; Kim, Y.-J.; Chung, H.-T.; Yun, Y.-G.; Kang, T.-H.; Jeong, O.-S.; Kim, Y.-C. Scopoletin suppresses pro-inflammatory cytokines and PGE2 from LPS-stimulated cell line, RAW 264.7 cells. *Fitoterapia* **2004**, *75*, 261–266. [CrossRef]
48. Kolar, M.J.; Konduri, S.; Chang, T.; Wang, H.; McNerlin, C.; Ohlsson, L.; Härröd, M.; Siegel, D.; Saghatelian, A. Linoleic acid esters of hydroxy linoleic acids are anti-inflammatory lipids found in plants and mammals. *J. Biol. Chem.* **2019**, *294*, 10698–10707. [CrossRef]
49. Blainski, A.; Lopes, G.; de Mello, J. Application and analysis of the Folin Ciocalteu Method for the determination of the total phenolic content from *Limonium brasiliense* L. *Molecules* **2013**, *18*, 6852–6865. [CrossRef]
50. Silva Mathias, M.; Rodrigues de Oliveira, R. Differentiation of the phenolic chemical profiles of *Cecropia pachystachya* and *Cecropia hololeuca*. *Phytochem. Anal.* **2019**, *30*, 73–82. [CrossRef]
51. Le, A.V.; Parks, E.S.; Nguyen, M.H.; Roach, P.D. Improving the vanillin-sulphuric acid method for quantifying total saponins. *Technologies* **2018**, *6*, 84. [CrossRef]
52. Salama, A.; Kotb, R.; Shaker, R. Effect of treatment durability and coloration of coated cotton fabrics on antibacterial, UV-blocking, healing and anti-inflammatory properties. *J. Chem. Pharm. Res.* **2015**, *7*, 181–193.
53. Franyoto, Y.D.; Kusmita, L.; Mutmainah; Angrena, R.D. Total flavonoid content and formulation antioxidant cream stem of *Jatropha multifida* L. *J. Phys. Conf. Ser.* **2018**, *1025*, 012130. [CrossRef]
54. Pashmforosh, M.; Rajabi Vardanjani, H.; Rajabi Vardanjani, H.; Pashmforosh, M.; Khodayar, M.J. Topical anti-inflammatory and analgesic activities of *Citrullus colocynthis* extract cream in rats. *Medicina (B. Aires)* **2018**, *54*, 51. [CrossRef] [PubMed]