Research Article

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Composition and Bioactivities of n-Hexane Extract from Jatropha integerrima Aerial Parts in Vietnam

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ABSTRACT

This research aimed to evaluate the antioxidant, anti-inflammatory, and antimicrobial properties of the n-hexane fraction extracted from the aerial parts of Jatropha integerrima. Aerial parts of the plant were harvested from Quang Tri, Vietnam, and extracted with n-hexane. The chemical composition of the n-hexane fraction was analyzed using gas chromatography-mass spectrometry (GC-MS). Antioxidant potential was measured by the DPPH assay, anti-inflammatory effects were assessed through nitric oxide inhibition in LPS-activated RAW264.7 cells, and antimicrobial activity was determined using the minimum inhibitory concentration (MIC) method. GC-MS analysis revealed 27 compounds in the n-hexane fraction, representing 99.3% of the total. Major constituents included methyl palmitate (25.6%), methyl oleate (22.1%), methyl linoleate (12.8%), methyl stearate (12.1%), palmitic acid (5.2%), and stearic acid (4.2%). The n-hexane extract showed weak antioxidant activity (IC50 = 1624.64 ± 24.96 µg/mL), strong anti-inflammatory effects (IC50 = 185.91 ± 8.08 µg/mL), and moderate antibacterial activity against Staphylococcus aureus (MIC = 512 µg/mL). According to the results, there is hope for the semi-volatile and GC-MS-detectable lipophilic components in the J. integerrima n-hexane extract to be investigated further as potential antioxidant, anti-inflammatory, and antibacterial agents.

Keywords: *Jatropha integerrima,* antimicrobial, antioxidant, anti-inflammatory, GC-MS

1. Introduction

As a part of the Euphorbiaceae family, the *Jatropha* genus includes a wide range of plants that are distinguished by their rich phytochemical profiles and important biological activity. This genus, which includes approximately 175 species of shrubs and trees, is predominantly distributed across tropical and subtropical regions. The chemical richness and the biological efficacy of *Jatropha* species have garnered considerable scientific interest, positioning them as a valuable subject of study for various applications [1, 2].

Phytochemical constituents including diterpenoids, represent the most prominent class of compounds in *Jatropha* species, with notable examples such as jatrophone, curcusone B, jatrogrossidione, jatropholone, jatrophatrione, and jatropholone A and B. Numerous biological actions, such as antibacterial, anti-inflammatory, and anticancer effects, have been shown for these substances [1, 3]. Flavonoids in *Jatropha* species, including quercetin, kaempferol, isovitexin, apigenin, luteolin, and their glycosides, are well-documented for their antioxidant properties. These compounds also exhibit potential health benefits such as cardioprotective and anti-inflammatory effects [1, 4]. Alkaloids, such as jatrophine, jatropham, and curcain, present in *Jatropha* species, have shown various pharmacological activities, particularly anticancer and antimicrobial effects, highlighting their significance in drug development [1, 5]. The antioxidant properties of phenolic acids, such as caffeic acid, gallic acid, and *p*-hydroxybenzoic acid, are well-known and are found in many *Jatropha* species [1, 6]. These acids have potential therapeutic applications in preventing oxidative stress-related diseases. Essential oils derived from *Jatropha* species contain a variety of compounds, including anethole, caryophyllene, eugenol, germacrene D, humulene, limonene, methyl eugenol, α-copaene, α-pinene, β-myrcene, and β-selinene [7-15]. Additionally, other notable compounds include lignans (hinokinin, cubebin, epiyangambin, gadain), tannins (ellagic acid, tannic acid), saponins, phorbol esters, and curcacycline A [1, 16].

Moreover, the biological activities of the *Jatropha* genus are extensive, encompassing anti-inflammatory, anticancer, antimicrobial, and antioxidant properties. Extracts from species such as *J. curcas* and *J. gossypiifolia* have demonstrated significant inhibition of inflammatory mediators, indicating potential therapeutic use in treating inflammatory diseases [1]. The anticancer properties of *Jatropha* species have been extensively studied, with diterpenoids such as jatrophone and curcusone B showing cytotoxic effects against various cancer cell lines [17-19]. Additionally, Jatropha species exhibit potent antibacterial qualities against a range of pathogens, such as bacteria, fungi, and viruses [7, 12, 20-23]. Their high flavonoid and phenolic acid content is principally responsible for these species' antioxidant qualities since they have the ability to reduce oxidative stress and scavenge free radicals, potentially avoiding a number of chronic diseases [12, 23, 24]. Furthermore, studies have been conducted on the various chemical compositions and noteworthy biological properties of essential oils derived from *Jatropha* species, such as their antifungal, antioxidant, antibacterial, anti-inflammatory, cytotoxic, and insecticidal characteristics [1, 25].

In conclusion, the *Jatropha* genus has a wealth of phytochemicals with important and varied biological activity, highlighting its potential for use in treatment. This work intends to bridge the divide by analyzing the chemical composition of the n-hexane fraction from *J. integerrima* aerial parts and exploring its biological characteristics.

2. Materials and Methods

2.1. Plant materials

In January 2024, *J. integerrima* aerial parts were taken from Quang Tri, Vietnam, at the following coordinates: N 17° 03.291', E 107° 04.344'. Dr. Anh Tuan Le of the Vietnam National Museum of Nature, Mien Trung Institute for Scientific Research, carried out the botanical identification. The University of Agriculture and Forestry, Hue University, Vietnam, has deposited a voucher specimen, designated JIL-2024, for use in future research.

2.2. Extraction procedure

The aerial parts of *J. integerrima* were ground, dried, and extracted using *n*-hexane at room temperature. A total of 6 kg of powdered material was processed, resulting in 70.0 g of a crude *n*-hexane extract (JH), which was obtained by evaporating the supernatant at 40°C under reduced pressure. The crude extract was further fractionated using solid-phase extraction

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on a silica gel column with a gradient elution system of *n*-hexane and acetone (60/1, 40/1, 20/1, 10/1, 5/1, v/v), yielding six fractions labeled JH1–JH6. From JH1 (10 g), a 1 mg portion was dissolved in *n*-hexane to produce a 100 mg/mL solution. The samples were placed in vials and filtered via 0.45 µm filters in order to maintain their semi-volatile compound and stability for further investigation.

2.3. The GC-MS analysis

A Shimadzu GCMS-QP2010 Plus system with an Equity-5 capillary column was used to perform the GC-MS analysis [26, 27]. Helium was employed as the carrier gas at a flow rate of 1.5 mL/min, with the temperature program starting at 60 °C and increasing up to 280 °C. The mass spectrometer covered a mass range of 40–500 amu while operating at an ionization voltage of 70 eV. Retention indices and reference data were used for chemical identification, while peak regions in the total ion chromatogram were used for quantification [28]. This analysis successfully identified and characterized the chemical constituents of *J. integerrima*'s *n*-hexane fraction, aiding future research.

2.4. DPPH radical scavenging activity

To measure antioxidant activity, a modified version of the DPPH radical scavenging experiment was used [29-31]. The research sample was dissolved in methanol and diluted to various concentrations for the assay, with L-ascorbic acid serving as the reference control. A 0.25 M DPPH solution in methanol was prepared as the reagent. The sample solution and DPPH reagent were combined in equal amounts in a 96-well plate, and the mixture was incubated for 30 minutes at room temperature. The radical scavenging activity was determined by measuring absorbance at 517 nm. The percentage of scavenging activity was computed, and curve-fitting software was used to estimate the IC_{50} value, which is the concentration required to neutralize 50% of the DPPH radicals.

2.5. Detection of nitric oxide production in the n-hexane fraction

RAW 264.7 cells were employed in this study, seeded into 96-well plates at a density of approximately 5×10^4 cells per well [26, 27, 29, 30]. After incubating for 24 hours at 37°C with 5% CO_2, the medium was replaced with FBS-free DMEM, followed by an additional 3-hour incubation. The cells were pretreated with varying concentrations of *n*-hexane extract (0.8–200 µg/mL) for two hours and then stimulated with 1 µg/mL lipopolysaccharide (LPS) for 24 hours. Nitric oxide (NO) production was measured as nitrite (NO_2^-) levels using the Griess reagent in a colorimetric assay, with absorbance readings at 540 nm. A positive control was employed, which was dexamethasone. Utilizing a sodium nitrite ($NaNO_2$) standard curve, NO inhibition was measured and IC_{50} values were computed by curve-fitting software. The mean ± standard deviation was used to express the data from three separate experiments.

Cell source and culture

The RAW 264.7 macrophage cell line was generously provided by Prof. Dr. Domenico Delfino from the University of Perugia, Italy. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), which was enhanced with 1.0 mM sodium pyruvate, 10% fetal bovine serum (FBS; Gibco), 10 mM HEPES, and 2 mM L-glutamine. Every 3 to 5 days, cell subculturing was carried out at a 1:3 ratio. A Thermo Scientific Heracell 150i incubator (USA) was used to incubate the cultures at 37 °C in a humidified environment with 5% CO_2.

2.6. Antimicrobial assay

Pathogenic strains obtained from the American Type Culture Collection (ATCC), specifically Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, were utilized in this study [27, 32-34]. At 37 °C, the bacteria were grown on Muller-Hinton Agar plates. Ethanol was used to dissolve the extract samples at concentrations ranging from 4 to 256 µg/mL. Each well of the microplate was prepared by adding 20 µL of the extract to 180 µL of the bacterial suspension (10^6 CFU/mL), followed by incubation at 37 °C. The lowest concentration that stops bacterial growth, known as the minimum inhibitory concentration (MIC), was found by measuring optical density at 600 nm. Tetracycline and streptomycin served as the reference drugs for Gram-positive and Gram-negative bacteria, respectively, and the assays were run in triplicate.

3. Results and Discussion

The GC-MS analysis of the *n*-hexane fraction from the aerial parts of *J. integerrima* revealed a diverse

chemical profile, with 27 compounds identified, accounting for 99.3% of the total GC-MS-detectable lipophilic content (Table 1, Figure 1). Ester compounds (83.8%) and acid compounds (13.1%) were the primary chemical groups identified. Furthermore, there were lower concentrations (1.0%) of oxygenated sesquiterpenes (0.7%), triterpene compounds (0.6%), oxygenated diterpenes (0.4%), alkane compounds (0.4%), aldehyde compounds (0.2%), and vitamin E constituents (0.2%) (Table 1, Figure 1). Among the ester compounds, the dominant constituents were methyl palmitate (25.6%), methyl oleate (22.1%), methyl linoleate (12.8%), and methyl stearate (12.1%). Other significant esters included ethyl linoleate (2.8%), ethyl palmitate (1.8%), methyl behenate (1.3%), methyl linolenate (1.1%), methyl margarate (0.9%), methyl arachidate (0.8%), ethyl stearate (0.7%), methyl palmitoleate (0.5%), methyl myristate (0.4%), methyl pentadecanoate (0.4%), hexahydrofarnesyl acetone (0.4%), and methyl labdanolate (0.1%). With acid compounds making up 13.0% of the *n*-hexane fraction, they were the second most abundant group. Among the class's prominent components were palmitic acid (5.2%), stearic acid (4.2%), linolenic acid (2.5%), and linoleic acid (1.1%) (Table 1). Additionally, compounds with concentrations exceeding 0.1% included oxygenated sesquiterpenes (0.7%), triterpene compounds (0.6%), oxygenated diterpenes (0.4%), alkane compounds (0.4%), aldehyde compounds (0.2%), and vitamin E constituents (0.2%). Esters and acids are present in significant quantities in the *n*-hexane fraction extracted from the aerial parts of *J. integerrima*, as confirmed by a detailed investigation. The chemical profile of these compounds suggests promising potential for various applications leveraging their bioactive properties.

Previous literature has reported the chemical profiles of various *Jatropha* species using GC-MS analysis. For instance, studies on *J. podagrica* identified 11 compounds in the seeds, accounting for 84.3% of the semi-volatile components, with palmitic acid (40.8%) and linoleic acid (16.6%) as the primary constituents [35]. Eleven chemicals totaling 59.9% of the semi-volatile components in the flowers were found. Lauric acid (13.8%), phenol (10.4%), and palmitic acid (17.8%) were the main constituents in the flowers [36]. GC-MS was used to examine the chemical makeup of the ethanol leaf extract of *J. gossypifolia*. The phytocomponents with the biggest peak areas were lanosterol (32.47%) and globulol (18.96%), out of the eighteen compounds found in the results [37]. Similarly, cyclotetracosane (10.1%), 9-hexacosene (9.4%), and hexadecanoic acid (3.9%) were among the chemicals found in the ethyl acetate extract of *J. zeyheri* [21].

Fourteen notable chemical components were also found in the methanolic leaf extract of *J. heynei*. The three most prevalent ones were phytol, hexadecanoic acid, and pentadecanoic acid [22]. Furthermore, thirty-four phytocomponents were discovered

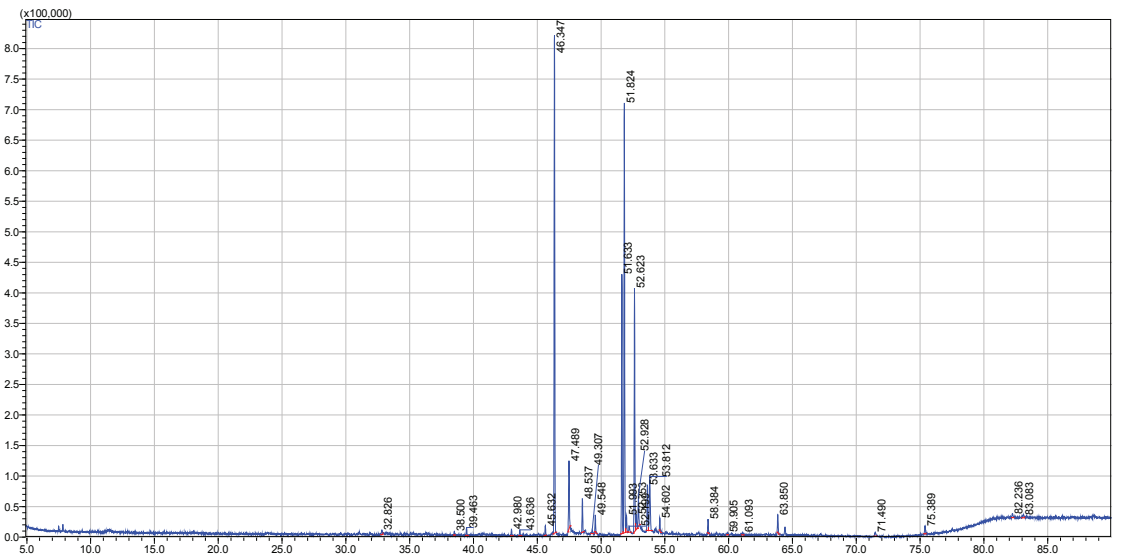

Figure 1. The GC chromatogram of *n*-hexane fraction (**JH1**) from *J. integerrima* aerial parts

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by GC-MS analysis of the methanolic leaf extract of *J. gossypifolia*. 2,4-heptadienal (*E,E*) (6.77%) and 1-monolinoleoylglycerol trimethylsilyl ether (9.58%) were the two main phytoconstituents [38]. Additionally, *β*-sitosterol (22.4%), 10-undecenoic acid octyl ester (16.36%), phytol (14.05%), and *β*-amyrin (10.50%) were among the phytocomponents identified by GC-MS analysis in the ethanol extract of *J. tanjorensis* leaves [39]. Research on the chemical composition of essential oils from different *Jatropha* species has shown some commonalities. The essential oils extracted from various regions of the *Jatropha* species exhibit notable variations in their chemical composition. As previously reported, GC-MS analysis was used to determine the essential oils from *J. integerrima* leaves and seeds. Pentadecanal (32.4%) and 1,8-cineole (11.2%) were the main constituents found in the leaf oil, whereas aliphatic hydrocarbons including *n*-pentacosane (13.6%), *n*-hexacosane (13.3%), and *n*-octacosane (12.3%) comprised the majority of the seed oil [7]. The essential oil composition of the roots of *J. ribifolia* revealed notable levels of *β*-pinene (9.16%), *β*-vatirene (8.34%), and *α*-gurjunene (6.98%) [8]. Palmitic acid (40.89%) and linoleic acid (16.64%) were the majority of the semi-volatile components found in *J. podagrica* seeds, whereas palmitic acid (17.87%) and lauric acid (13.81%) were present in the flowers [35]. Chemical examination of the essential oil of *J. neopauciflora* revealed the presence of ledene (14.29%), *m*-menthane (12.77%), linalyl acetate (12.03%), and 1,3,8-*p*-menthatriene (14.98%). In the methanol extract obtained from the dried aerial parts, there was 22.08% lupeol, 16.01% *β*-sitosterol, and 14.34% arabinol [10]. Neophytadiene (35.8%), phytol (23.1%), and *trans*-pinane (12.7%) were the constituents of the essential oil extracted from the leaves of *J. curcas* [11]. *J. curcas* leaves were found to contain *δ*-cadinene (9.6%), *α*-*epi*-cadinol (7.38%), and pulegone (5.95%) in additional research [12]. Oleic acid (42.8%) and linoleic acid (32.8%) were the two main fatty acids found in *J. curcas* seed oil [13]. Two compounds found in *J. gossypifolia* essential oils by GC-MS studies were linalool (9.81%) and phytol (33.40%) [14]. Heptadecanoic acid (35.81%), linoleic acid (24.09%), and oleic acid (7.4%) were the fatty acids found in the seed oil [40]. Many factors, including species, geographic location, extraction techniques, plant parts used, growing circumstances, and maturity stage at harvest, greatly affect the chemical composition of *Jatropha* essential oils. These variations contribute to the distinctive biological characteristics of each *Jatropha* species, highlighting the potential of *J. integerrima* aerial parts and other species for various therapeutic applications.

In addition to its complex chemical composition, the *Jatropha* genus is notable for its biological activities, including antioxidant capacity. The antioxidant potential of *n*-hexane extract is typically assessed using the DPPH assay. For instance, with an IC_{50} value of 1624.64 ± 24.96 µg/mL, the *n*-hexane extract derived from *J. integerrima* aerial parts demonstrated moderate antioxidant activity. This value is similar to that of L-ascorbic acid, the positive control, which had an IC_{50} value of 7.78 ± 0.38 µg/mL. Given the relatively modest amounts of total phenolic components in the *n*-hexane extract, this limited efficacy may be explained. For the DPPH radical scavenging test, *n*-hexane extract of *J. integerrima* aerial parts which are mainly made up of non-polar substances like fatty acid methyl esters (like methyl palmitate and methyl oleate) and free fatty acids (such palmitic acid and linoleic acid) was dissolved in methanol. These molecules may have had restricted solubility in methanol, a polar solvent, because of their non-polar character. This might have caused the extract to dissolve partially, which could have understated the antioxidant activity in the DPPH test. To increase the solubility of the lipophilic components and give a more precise evaluation of their antioxidant capability, future research could investigate the use of different solvents, such as a combination of methanol and a non-polar co-solvent (such as dichloromethane or *n*-hexane). Comparable research on *J. gossypifolia* has revealed a noteworthy antioxidant activity, wherein the essential oils of the stem exhibit greater antiradical strength (IC_{50} 0.07 mg/mL) in scavenging DPPH radicals than the leaves (IC_{50} 0.32 mg/mL) and *β*-carotene (IC_{50} 1.62 mg/mL) [14]. Additionally, the DPPH and reducing power tests revealed that the essential oil of *J. curcas* leaves exhibited moderate antioxidant activity, with IC_{50} values of 314 µg/mL and 298 µg/mL, respectively [12]. These findings highlight the potential of *Jatropha* essential oils as natural antioxidants, with varying effectiveness across species and plant parts. The ethyl acetate extract from *J. zeyheri* roots showed IC_{50} values of 19.66 µg/mL (DPPH), 22.63 µg/mL (ABTS), and 1.82 µg/mL (Fe^{2+}) [21]. In another study, the ethanolic extract exhibited the highest DPPH radical scav-

Table 1. Chemical composition of the *n*-hexane fraction (JH1) from the aerial parts of *J. integerrima*

No.	RT	Compound	RI_E	RI_L	Concentration (%)
1	32.83	*α*-Agarofuran	1548	1548	0.4
2	38.50	Germacrone	1697	1693	0.3
3	39.46	Methyl myristate	1724	1722	0.4
4	42.98	Methyl pentadecanoate	1825	1817	0.4
5	43.64	Hexahydrofarnesyl acetone	1844	1845	0.4
6	45.63	Methyl palmitoleate	1903	1896	0.5
7	46.35	Methyl palmitate	1925	1921	25.6
8	47.49	Palmitic acid	1961	1959	5.2
9	48.54	Ethyl palmitate	1993	1992	1.8
10	49.31	Octadecanal	2018	2013	0.2
11	49.55	Methyl margarate	2025	2022	0.9
12	51.63	Methyl linoleate	2093	2095	12.8
13	51.82	Methyl oleate	2099	2103	22.1
14	51.99	Methyl linolenate	2105	2105	1.1
15	52.20	Phytol	2112	2117	0.4
16	52.62	Methyl stearate	2126	2126	12.1
17	52.75	Linoleic acid	2130	2132	1.1
18	52.93	Linolenic acid	2136	2137	2.5
19	53.63	Ethyl linoleate	2160	2159	2.8
20	53.81	Stearic acid	2166	2162	4.2
21	54.60	Ethyl stearate	2193	2189	0.7
22	58.38	Methyl arachidate	2327	2329	0.8
23	59.91	Methyl labdanolate	2383	2381	0.1
24	63.85	Methyl behenate	2525	2530	1.3
25	75.39	Squalene	2827	2829	0.6
26	82.24	Untriacontane	3095	3100	0.4
27	83.08	*d*,*α*-Tocopherol	3131	3111	0.2
		Total			99.3
		Ester compounds			83.8
		Acid compounds			13.0
		Oxygenated sesquiterpenes			0.7
		Triterpene compounds			0.6
		Oxygenated diterpens			0.4
		Alkane compounds			0.4
		Aldehyde compounds			0.2
		Vitamin E constituents			0.2

RT: Retention time, RI_E: Retention indices relative to *n*-alkanes (C_7-C_{40}) on Equity-5 column, RI_L: Retention indices from the Adams book [28].

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enging activity with an IC_{50} of 0.72±0.03 mg/mL. The methanolic extract had the highest ferric ion reduction at 46.23±1.10 µg Equivalent Antioxidant Activity (EAA)/g. Compared to the Ferric Reducing Antioxidant Power (FRAP) method, the ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) method showed higher reducing power for the water-ethanolic extract, with a value of 0.49±0.11 mol Equivalent Trolox (ET)/g [23]. A study on *J. curcas* in Morocco found that its essential oil demonstrated moderate antioxidant activity, with IC_{50} values of 314 µg/mL in the DPPH radical scavenging test and 298 µg/mL in the reducing power test [12]. The ability of *J. multifida* leaf extracts to prevent protein (albumin) denaturation was tested at 660 nm. The water-ethanolic extract had the highest inhibition (97.31 ± 0.35%) at 1000 µg/mL, followed by the methanolic extract (95.35 ± 1.05%). The dichloromethane extract had the lowest inhibition (80.64 ± 1.32%). Although the methanolic extract had the lowest IC_{50} (2.24 ± 0.03 µg/mL), compared to the water-ethanolic extract (7.12 ± 0.24 µg/mL). The dichloromethane and acetone extracts had the highest IC_{50} values of 262.17 ± 4.07 µg/mL and 232.51 ± 3.96 µg/mL, respectively [23]. All things considered, the genus *Jatropha* offers a wealth of phytochemicals with diverse and significant biological roles, including antioxidant properties. Varying *Jatropha* species and plant sections have varying chemical compositions, which add to their unique biological traits and emphasize their potential for a range of medicinal uses.

This work used the DPPH assay to evaluate the antioxidant activity of the *n*-hexane extract from the aerial portions of *J. integerrima*. Despite being widely utilized, the DPPH technique does not capture additional antioxidant processes and mostly reflects radical scavenging. Other tests, including ABTS and FRAP, were not carried out due to time and resource constraints. These techniques ought to be used in future research for a more thorough assessment of antioxidants. The generation of nitric oxide (NO), which is crucial for inflammatory responses, can be excessive and cause a number of health problems. Therefore, inhibiting NO accumulation is seen as a promising therapeutic approach. The efficacy of the *n*-hexane fraction from *J. integerrima* aerial parts to prevent NO generation in LPS-induced RAW264.7 macrophage cells was investigated; results are shown in Table 3. At concentrations near the IC_{50} (150 µg/mL), cell viability remained 100.28 ± 1.48%, and at 200 µg/mL, it was 97.69 ± 1.04%, indicating that the observed NO inhibition was not due to cytotoxic effects on RAW264.7 cells. Notably, the *J. integerrima* aerial parts fraction showed significant NO production inhibition, with an IC_{50} of 185.91 ± 8.08 µg/mL. The positive control, dexamethasone, showed an IC_{50} of 13.22 ± 1.36 µg/mL. Another study investigated the anti-inflammatory effects of *Jatropha integerrima* leaf extract using a mouse paw edema model. Oral administration of the extract at a dose of 400 mg/kg resulted in a 63.09% reduction in paw swelling, surpassing the effect of the standard drug indomethacin. Additionally, the extract significantly lowered the levels of prostaglandin E2 (PGE2), nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), and protein kinase C (PKC) [24]. These studies underscore the potential therapeutic benefits of *Jatropha* species in managing inflammation and oxidative stress.

In the antimicrobial assessment detailed in Table 4, the *n*-hexane fraction from *J. integerrima* aerial parts displayed limited activity against the Gram-positive bacterium *Staphylococcus aureus*, with a MIC of 512 µg/mL. It was ineffective against the Gram-negative bacterium *E. coli*, though. Likewise, *J. integerrima*'s essential oils from the leaves and seeds showed marginal antibacterial activity against Gram-positive bacteria like *Bacillus cereus* and *Staphylococcus aureus*, with MIC of up to 625 µg/mL. However, they had no effect on the Gram-negative strain of *Pseudomonas aeruginosa* (MIC = 1250 µg/mL) [7]. The essential oil extracted from *J. curcas* leaves, on the other hand, demonstrated strong antibacterial action against a wider range of microbes. Six bacteria (*E. Coli, P. aeruginosa, K. pneumoniae, S. aureus, M. luteus,* and *B. cereus*) and three fungus (*A. niger, Penicillium sp.,* and *R. rubra*) were all efficiently suppressed by it [12]. Further studies on *J. podagrica* rootwood and root bark extracts (*n*-hexane, chloroform, and methanol) revealed broad-spectrum antibacterial activity against 18 organisms at 20 mg/mL. The *n*-hexane extracts exhibited the strongest activity, with the *n*-hexane root bark extract showing the highest potency, comparable to gentamycin and more effective against *S. aureus* and *B. cereus*. Additionally, the *n*-hexane root bark extract and the *n*-hexane and methanol rootwood extracts demonstrated moderate antifungal activity against *C. albicans* [20]. *J. zeyheri* root ethyl acetate extract showed the lowest MIC, 40 µg/mL against *K. pneumoniae* and 80 µg/

Table 2. IC_{50} values for DPPH radical scavenging activity of the *n*-hexane fraction (JH1) from the aerial parts of *J. integerrima*

Concentration (µg/mL)	DPPH scavenging (%)			
	JH1		L-Ascorbic acid[b]	
	Average	SD	Average	SD
2000	65.24	1.21	-	-
1000	27.46	1.54	-	-
500	21.45	1.10	-	-
100	8.65	0.89	91.23	1.58
20	1.57	0.16	73.88	1.22
4	0.96	0.06	33.20	1.29
0.8	-	-	12.60	0.81
IC_{50}[a]	208.37 ± 10.67		7.60 ± 0.31	

[a]IC_{50}: Scavenging Concentration at 50% – concentration that neutralizes 50% of DPPH free radicals; [b]Positive control; SD: Standard Deviation.

Table 3. *In vitro* anti-inflammatory activity of the *n*-hexane fraction (JH1) from the aerial parts of *J. integerrima*

Concentration (µg/mL)	JH1		Dexamethasone[b]	
	NO inhibition rate (%)	Viability rate (%)	NO inhibition rate (%)	Viability rate (%)
200	52.98±1.94	97.69±1.04	-	-
150	42.22±1.16	100.28±1.48	-	-
100	32.80±1.65	103.13±2.09	86.88±1.88	93.82±1.35
20	10.33±1.06	-	53.49±1.19	99.23±1.00
4	2.28±0.23	-	42.47±0.88	-
0.8	-0.90±0.06	-	31.62±1.05	-
IC_{50}[a]	185.91±8.08		13.22±1.36	

[a]IC_{50} : concentration that inhibits 50% of cell growth; [b]Positive control.

mL against *C. albicans*, *S. aureus*, and *M. hominis* [21]. The methanolic leaf extract of *J. heynei* demonstrated good in vitro antibacterial activity, with a zone of inhibition of 14±0.57 mm against *S. aureus* and 10±0.25 mm against *P. aeruginosa* [22]. Extracts from *J. multifida* (methanol, acetone, dichloromethane, ethanol, and water-ethanol) demonstrated bactericidal effects, with Minimum Bactericidal Concentration (MBCs) ranging from 22.67 mg/mL (e.g., *S. aureus*, *S. enteritidis*) to 47.61 mg/mL (*E. coli*), also showing activity against meat-isolated strains like *S. equorum* and *S. saprophyticus* [23].

Previous phytochemical analyses of polar extracts from seeds or leaves of *Jatropha* species, including *J. curcas* and *J. gossypifolia*, have identified phenolic chemicals, phorbol esters, and diterpenoids as major components. On the other hand, our investigation is the first to describe the *n*-hexane fraction from *J. integerrim*a's aerial parts, and it reveals a distinct profile that is dominated by fatty acid esters (such as methyl palmitate and methyl oleate). The non-polar solvent employed and the particular plant part under investigation may be to blame for this discrepancy. The antibacterial, anti-inflammatory, and antioxidant

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Table 4. Antimicrobial activity of the *n*-hexane fraction (JH1) from the aerial parts of *J. integerrima*

Microbial strains		MIC: µg/mL		
		JH1	Streptomycin	Tetracycline
Gram (+)	*S. aureus*	512	8	-
Gram (–)	*E. coli*	-	-	4

properties shown in this study point to a new finding and suggest for more research into the fraction's active ingredients.

4. Conclusion

This study represents the first comprehensive analysis of the phytochemical composition, antioxidant, anti-inflammatory, and antibacterial properties of the *n*-hexane fraction from the aerial parts of *J. integerrima* collected in Vietnam. Using GC-MS, we identified 27 compounds constituting 99.3% of the semi-volatile content, with methyl palmitate (25.6%), methyl oleate (22.1%), and methyl linoleate (12.8%) as the dominant constituents. This chemical profile differs from previous studies on *Jatropha* species, which primarily focused on polar extracts and reported higher proportions of diterpenoids and phorbol esters, whereas our *n*-hexane fraction is enriched with fatty acid esters. The fraction exhibited weak antioxidant activity (IC_{50}: 1624.64 ± 24.96 µg/mL), likely due to the lipophilic nature of the compounds and limited solubility in the DPPH assay, but demonstrated strong anti-inflammatory effects (IC_{50}: 185.91 ± 8.08 µg/mL) and moderate antibacterial activity against *Staphylococcus aureus* (MIC: 512 µg/mL). These results demonstrate a new use of the *n*-hexane fraction, specifically its strong anti-inflammatory properties, which have not been documented for these plant parts before utilizing a non-polar solvent extraction method. This work lays the foundations for further investigations to identify active ingredients and investigate their potential as remedies for bacterial infections and inflammation.

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Conflicts of Interest

The author has no conflicts of interest, financial or otherwise to declare.

Statement of Contribution of Researchers

Conceptualization: T. V . P., L. T. L., L. T. L., D. V. H., and H. N. T. H.; Data collection: T. V. P., L. T. L., L. T. L., D. V. H., and H. N. T. H.; Formal analysis: T. V. P. and H. N. T. H.; Investigation: T. V. P. and H. N. T. H.; Methodology: T. V. P., L. T. L., L. T. L., D. V. H., and H. N. T. H.; Resources: T. V. P. and H. N. T. H.; Writing original draft: T. V. P. and H. N. T. H.; Writing review & editing: T. V. P., L. T. L., L. T. L., D. V. H., and H. N. T. H.

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