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Supercritical Carbon Dioxide Extraction of Fatty Acids Compositions from *Epiphyllum oxypetalum* (DC.) Haw. Leaves Oil

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In traditional medicine, *Epiphyllum oxypetalum (E. oxypetalum)* is used to treat and promote wound healing and general health management. Due to the drawbacks of conventional extraction methods, such as using toxic solvents, supercritical fluid extraction using carbon dioxide was proposed as a green extraction method for better results. This research aims to extract the *E. oxypetalum* leaves oil using supercritical fluid extraction with carbon dioxide as the solvent and to determine the fatty acids composition in the extract of *E. oxypetalum* leaves oil. The extract of *E. oxypetalum* leaves oil was obtained via supercritical fluid extraction using carbon dioxide due to non–toxic, lower temperature, non–flammable, economical, and readily available. To identify the ideal operating conditions, the extraction was carried out at different temperatures (40 and 60 °C) and pressures (20 and 30 MPa) for one hour. At a temperature of 40 °C and a pressure of 30 MPa, the maximum oil yield percentage (3.2 ± 0.20 %) was obtained. Gas chromatography mass spectrometry (GC–MS) analysis revealed that the extract *E. oxypetalum* leaves oil contained six fatty acids compounds which are oleic acid (30.70 mol %), *n*–hexadecanoic acid (18.43 mol %), linoelaidic acid (10.27 mol %), myristic acid (6.56 mol %), dodecanoic acid (3.99 mol %), and phytol (3.41 mol %). The fatty acids composition presented in the extract of *E. oxypetalum* leaves oil have shown possible to be useful for subsequent applications in health and industrial uses.

Keywords: *Epiphyllum oxypetalum*; bakawali; GC–MS; supercritical fluid extraction; fatty acids; carbon dioxide

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The Cactaceae family member *Epiphyllum oxypetalum* (DC.) Haw. (*E. oxypetalum*) is regarded as one of the best–known *Epiphyllum* species. Other names for it include Wijayakusuma, Queen of Night, Brahmakamal, Bakawali, and Dutchman's Pipe. *E. oxypetalum* is a tropical plant that is used traditionally for the treatment of wound healing and improving hydration of the skin [1–3]. Upendra et al. [4] reported that the nutritive values of the extract of *E. oxypetalum* leaves showed a significant presence of fatty acids (4.6 mg/g), proteins (14 mg/g), and vitamins (0.18 mg/g), when the absence of carbohydrates was discovered. According to reports in Upendra et al. [4] work, the steroids, glycosides, saponins, phenolic, tannins, terpenoids, and resins compounds were detected using the gas chromatography mass spectrometry (GC–MS) analysis in the extract of *E. oxypetalum* leaves. Meanwhile, Dandekar et al. [2] reported that the extract of *E. oxypetalum* leaves contained various types of phyto-chemical constituents, including megastigmatrienone, cycloocta–1,3,6–triene, 2,3,5,5,8,8–hexamethyl, 4–((1e)–3–hydroxy–1–propenyl)–2–methoxyphenol, 2,5–dihydroxy–4–isopropyl–2,4,6–cycloheptatrien–1–one, stigmasterol, cholesta–22,24–dien–5–ol, 4,4–dimethyl, 22–stigmasten–3–one, heptacosane, 2–methyl–octadecane, 2–methyl–eicosane, hentriacontane, stig mast–4–en–3–one, testosterone cypionate, and hentria contane. As reported, the majority of the phyto-chemical constituents present in the *E. oxypetalum* leaves were found to be steroids [2]. Mahmad et al. [3] also reported that the extract of *E. oxypetalum* leaves contained fatty acids (dodecanoic acid and *n*–hexa decanoic acid) and phenolic compounds (2–methoxy–4–vinylphenol and vanillyl alcohol). From the results, it could be concluded that the extract of *E. oxypetalum* leaves contains various bioactive compounds with various medicinal properties.

In addition, Dandekar et al. [5] studied the anti–inflammatory of the extract of *E. oxypetalum* leaves by using *in vitro* method, which included human red blood cell membrane stabilization and inhibition of protein denaturation method with albino Wistar rats. The percentage inhibition was seen

maximum in 300 µg/ml concentration of alcohol, thus, proving that the extract of *E. oxypetalum* leaves exhibited anti–inflammatory activity. Besides, *E. oxypetalum* leaves were reported to exhibit anti–bacterial properties against *Staphylococcus aureus,* a common pathogen that slows the healing process of wounds [5]. Due to the anti–inflammatory and anti–bacterial properties of the extract of *E. oxypetalum* leaves, Dwita et al. [1] assessed the wound healing activity using the incision model. The results showed significant wound healing progress in diabetic mice treated with *E. oxypetalum* leaves extract ointment, as assessed by wound contraction, number of macrophages, and fibroblast count. The data also showed that topical application of 96 % of *E. oxypetalum* leaves accelerated the wound healing time in diabetic mice. With that, the extract of *E. oxypetalum* was proven to be anti–inflammatory and antioxidant [2–3]. Other than that, the fatty acids compound such as 2–methylnonadecane (volatile heterocyclic hydrocarbon), which contains hydrogen–donating characteristics of its hydroxyl groups, could function as an antioxidant compound [2]. Antioxidants are compounds that are becoming more significant due to their capacity to prevent oxidative stress–related damage to the body and improve skin hydration [6].

To acquire a high concentration of target compounds, it is essential to choose appropriate extraction procedures. Conventional extraction methods, such as those that employed organic solvents or steam distillation, run a significant amount of time (hours to days) and used a substantial quantity of the solvent for the extraction process [7]. Hexane may be used to extract the oil from the leaves of *E. oxypetalum*, although it is a hazardous solvent [7]. Ergo, this work proposed an alternative method of extraction, which is supercritical fluid extraction with carbon dioxide. It has potential to produce extracts that can be performed at low temperatures, which is essential to conserve the quality of thermosensitive products. The *E. oxypetalum* leaves were extracted via supercritical fluid extraction with carbon dioxide since it is selectivity, non–flammable, non–toxic, and economical from the extracted materials [8–9]. Moreover, supercritical fluid extraction uses carbon dioxide as a green solvent that operated at low temperatures lower than 100 °C to prevent the degradation of thermally labile compounds in the extraction. This method also has higher diffusivity and solubility than conventional extraction methods [9].

GC–MS is a combined analytical technique to determine and identify compounds in the *E. oxypetalum* leaves oil. GC–MS plays an essential role in the phyto-chemical constituents analysis of medicinal plants containing biologically active components. GC–MS is one of the optimum, rapid, and most accurate techniques to detect various compounds, including alcohols, alkaloids, nitro compounds, esters, steroids, organic acids, long–chain hydrocarbons, and amino acids that require a low volume of plant extracts [9]. This work aims to extract the *E. oxypetalum* leaves oil using supercritical fluid extraction with carbon dioxide as the solvent and to determine the fatty acids composition in the extract of *E. oxypetalum* leaves oil. To date, no reported work uses supercritical fluid extraction with carbon dioxide for *E. oxypetalum* leaves oil studies. In this work, the extract of *E. oxypetalum* leaves oil was achieved with varying pressures (20 and 30 MPa) and temperatures (40 and 60 °C) based on the experimental domain of supercritical phase extraction in pressure–density diagram for carbon dioxide [10]. The extract of *E. oxypetalum* leaves oil has been used to study the percentage of oil yield and fatty acids composition using GC–MS analysis. Therefore, this work has potential to prove the fatty acids composition in the extract of *E. oxypetalum* leaves oil, which it can be beneficial for further usage in industry utilization.

EXPERIMENTAL

Chemicals and Materials

All chemicals used were analytical grade. Carbon dioxide of 99.99% purity (Kras, Instrument and Services, Johor, Malaysia) was used as the primary solvent for the extraction. The *E. oxypetalum* leaves were collected from botanical gardens in Ipoh, Perak, Malaysia. The *E. oxypetalum* leaves were washed with distilled water and dried at 40 °C in the oven for a week. The *E. oxypetalum* leaves were ground in a mechanical grinder to have a fine powder. Then, until required, the dried *E. oxypetalum* leaves were kept at 4 °C in an airtight container.

Preparation *E. oxypetalum* Leaves Oil for Super-critical Fluid Extraction with Carbon Dioxide

The setup, as shown in Figure 1, was located in the Centre of Lipids Engineering and Applied Research (CLEAR), Faculty of Chemical Engineering, Universiti Teknologi Malaysia (UTM). The supercritical fluid extraction with carbon dioxide system consisted of 50 mL extraction vessel, a high–pressure pump (Super-critical 24, Lab Alliance), a modifier pump (Series II Pump, Lab Alliance), and carbon dioxide. The independent factors were pressure and temperature. The pressures applied were 20 and 30 MPa, and the temperatures were 40 and 60 °C within an hour.

While the input (B) and exit (J) valves were closed, the thick steel tube vessel (G) carrying 5 g of the *E. oxypetalum* leaves (I) was inserted vertically into the extractor (H) of supercritical fluid extraction with carbon dioxide. The system's input valve (B) was opened as soon as the system reached the requisite temperature during the heating procedure. The pump (D) continually pumps carbon dioxide (3.8 mL/min) from the storage tank (A) through the system to the input valve to reach the necessary pressure (B). After that, the extract of *E. oxypetalum* leaves oil was collected for around 30 minutes while the outlet (J) and exit valve (K) were opened. The oil yields were obtained after the extraction procedure was complete

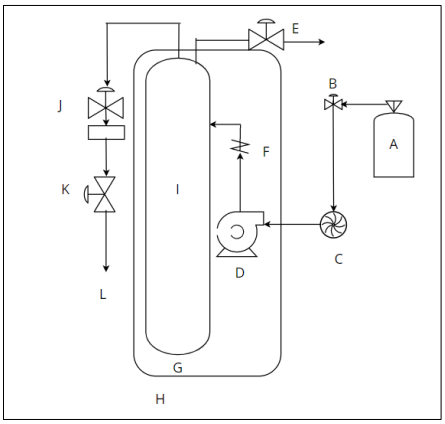


Figure 1. The schematic design of the supercritical fluid extraction with carbon dioxide system unit for the extract of *E. oxypetalum* leaves oil: (A) carbon dioxide inlet tank, (B) carbon dioxide inlet valve, (C) cooler, (D) pump, (E) vent valve, (F) pre–heat coil, (G) vessel, (H) extractor, (I) the extract of *E. oxypetalum* leaves oil, (J) outlet valve, (K) exit valve, (L) sample collector.

since the extraction vessel depressurized when the inflow valve (B) was closed, and the outlet valves (J, K) were opened. The uncrude oil was removed from the vessel (G) and weighed for calculations. The oil from the extract of *E. oxypetalum* leaves was weighed before and after each extraction. The final oil from the extraction of *E. oxypetalum* leaves was discovered to be an aqueous phase and waxy–state oil. Using a rotary evaporator with a 60 °C water bath, water in the aqueous phase extract was evaporated. The extract of *E. oxypetalum* leaves oil was evaporated until a steady weight was achieved. Every experiment was measured in triplicate to guarantee data dependability and precision [12].

Determination of Oil Yield for the Extract of *E. oxypetalum* Leaves Oil

The percentage of oil yield was calculated by taking into account the mass of the extract of *E. oxypetalum* leaves oil, and the mass of the *E. oxypetalum* leaves used for oil extraction by using Eq. (1) [13].

$$\text{Oil yield}\,(\%) = \frac{\text{Mass of the extract of } E.oxypetalum \text{ leaves oil}}{\text{Mass of } E.oxypetalum \text{ leaves}} \text{ x } 100 \qquad (1)$$

GC–MS Analysis for Fatty Acids Composition Determination of the Extract of *E. oxypetalum* Leaves Oil

The GC–MS analysis was carried out on the extract of *E. oxypetalum* leaves oil that had been diluted. To examine the metabolite composition, the extract of *E. oxypetalum* leaves oil was carried out in the GC–MS analysis. An Agilent 7890 apparatus was used for the GC, while a Joel Accu TOF GCV analyzer was used for the MS. The carrier gas was the inert gas helium (99.99 %) at a flow rate of 1 ml/min. A polyethylene glycol (BP–20) column of 30 mm by 0.25 mm by 0.25 m was employed. The method was conducted using an initial temperature of 50 °C, an injector temperature of 250 °C, and a temperature flow rate of 10 °C/min. The experiments lasted 50 minutes with a final temperature of 280 °C.

Mass Spectral Interpretation and Identification of Fatty Acids Composition in the Extract of *E. oxypetalum* Leaves Oil

The National Institute of Standards and Technology (NIST) databases were used to determine the extract of *E. oxypetalum* leaves oil. Based on the molecular formula and molecular weight, the structure of the components in the extract of *E. oxypetalum* leaves oil was confirmed.

RESULTS AND DISCUSSION

Percentage Oil Yield of the Extract of *E. oxypetalum* Leaves Oil with Different Temperatures and Pressures

The percentage oil yield of the extract of *E. oxypetalum* leaves oil was obtained by the influence of different pressures and temperatures, as shown in Table 1. Based on the results, the optimum percentage oil yield of the extract of *E. oxypetalum* leaves oil was 3.2 ± 0.20 % (sample 3) with a temperature of 40 °C and pressure of 30 MPa. The percentage oil yield of the extract of *E. oxypetalum* leaves oil increased as the pressure increased from 20 to 30 MPa. Meanwhile, the percentage oil yield of the extract of *E. oxypetalum* leaves oil was the lowest in sample 1 (1.6 ± 0.13 %), 2 (1.5 ± 0.19 %), and 4 (1.6 ± 0.11%). In a supercritical fluid extraction with the carbon dioxide process, the effect of temperature on the extraction rate at constant pressure results from two mechanisms: (1) the increase in process temperature, which increased the solubility due to the enhancement of solute vapor pressure, and (2) the fall in solubility due to the decrease in solvent

Table 1. The percentage oil yield of the extract of *E. oxypetalum* leaves oil with different pressure and temperature based on supercritical fluid extraction with the carbon dioxide method

Sample no.	Pressure (MPa)	Temperature (˚C)	% Oil Yield ± SD
1	20	40	1.6 ± 0.13
2		60	1.5 ± 0.19
3	30	40	3.2 ± 0.20
4		60	1.6 ± 0.11

*SD: standard deviation

Table 2. ANOVA results for significance test

Source of Variation	Sum of squares (SS)	Degree of freedom (df)	Mean square (MS)	F–ratio	P–value
Between Groups	2092.71	2	1046.36	4.54	0.0433
Within Groups	2073.84	9	230.43		
Total	4166.55	11			

density. According to operating circumstances, the solvent power was characterized by supercritical fluid extraction with carbon dioxide density and affected by temperature [13]. According to theory, a more significant temperature may have led to the breakdown or rupture of the cell walls, which subsequently made it easier to remove the oil due to the larger surface area available for diffusion [7]. Generally, the higher the pressure, the greater the solvent power and the greater the extracted percentage oil yield in supercritical fluid extraction using carbon dioxide. As the result, the percentage oil yield of the extract of *E. oxypetalum* leaves oil was significantly enhanced under high pressure of 30 MPa (40 ºC).

Table 2 displays the outcomes of the one–way analysis of variance (ANOVA) that was performed. The study yielded a P–value of 0.0433, which has a lower than 0.05 possibility of occurrence by chance. The F–ratio was discovered to be higher than the F–distribution table's crucial value F2,9 of 4.26. Based on the evaluation of the ANOVA calculation and the P–value and F–ratio, the findings were shown to be statistically significant.

GC–MS Analysis of Fatty Acids Composition

Plants are the most significant source for identifying new compounds with potential medicinal applications that may be used in the production of medicines. GC–MS has been one of the most popular methods in chromatography technologies for separating phyto-chemical constituents in recent years [14]. Based on the percentage oil yield, sample 3 was selected as the optimum result and proceeded with GC–MS analysis. The presence of diverse phytochemical constituents from the dissolved extract of *E. oxypetalum* leaves oil revealed 17 peaks (sample 3) with different retention times, as shown in Figure 2. Meanwhile, Table 3 shows the phytochemical constituents found in the extract of *E. oxypetalum* leaves oil for sample 3. The peak heights showed the relative concentrations of components found in the extract of *E. oxypetalum* leaves oil and were compared to the primary library data. The chemical ingredients in the extract of *E. oxypetalum* leaves oil were identified by comparing them to an internal spectral database based on >80 % similarity index [2].

As shown in Table 3, dodecanoic acid, myristic acid, oleic acid, linoelaidic acid, *n*–hexadecanoic acid, and phytol compounds were commonly presented in the extract of *E. oxypetalum* leaves oil similar reported to Dandekar et al. [2] data. Table 4 shows the phyto-chemical constituents GC–MS data study of the extract of *E. oxypetalum* leaves oil proving the presence of fatty acids composition with the uses. Six different fatty acids compounds with chemical structures have been identified and listed in Table 4. The highest fatty acids compounds were oleic acid (30.70 mol %), followed by *n*–hexadecanoic acid (18.43 mol %) and linoelaidic acid (10.27 mol %). Other fatty acids compounds identified were myristic acid (6.56 mol %), dodecanoic acid (3.99 mol %), and phytol (3.41 mol %). The fatty acids composition of the extract of *E. oxypetalum* leaves oil was compared with previous studies found in the literature. However, hexanoic acid, ethanedioic acid, oxirane, [(hexadecyloxy) methyl]–, 1H–imidazole, 4,5–dihydro–2,4–dimethyl–, 2,6,10–dodecatrien–1–ol, 3,7,11–trimethyl–, allyl ethyl sulfide, 2–isopropoxyethylamine, and pentadecanoic acid were absent for sample 3 in the extract of *E. oxypetalum*

leaves oil. Four fatty acids compounds were absent, such as hexanoic acid, ethanedioic acid, 1H–imidazole, 4,5–dihydro–2,4–dimethyl– and pentadecanoic acid in the extract of *E. oxypetalum* leaves oil.

The extract of *E. oxypetalum* leaves oil has several biological activities as well as industrial uses. The presence of phytochemical constituents in the extract of *E. oxypetalum* leaves oil possibly indicated its numerous medicinal properties [16]. Oleic acid was the highest fatty acid compound in the extract of *E. oxypetalum* leaves oil. In the dietary, oleic acid was essential to the human body in cellular energy production and myriad metabolic functions [17]. Oleic acid lowered the risk of heart disease, reduced blood pressure, anti–obesity and anti–cancer agent [17–18]. As reported, oleic acid was beneficial when designing optimal dietary fat intake to counteract hyper-coagulability postprandial states and reduced post-prandial insulin levels by recovering peripheral insulin sensitivity. In cosmetics, oleic acid was used as a cleansing agent, antimicrobial, and texture enhancer. Oleic acid was a stable fatty acid used in various beauty products to enhance product absorption and increase the ability to lock in moisture [19]. It was also used as an emulsifying or solubilizing agent in aerosol products [20].

Dodecanoic acid and *n*–hexadecanoic acid, for instance, have antibacterial, anti–inflammatory, antioxidant, and antifungal properties [21–23]. The oil from the extract of *E. oxypetalum* leaves may be used as a secure and affordable herbal medicine for wound healing due to its antibacterial and anti–inflammatory effects [1]. It has been shown that most oxidizing molecules, such as singlet oxygen and other free radicals [2] associated with several diseases, were efficiently neutralized by flavonoids. The mucous membranes were affected by flavonoids' anti–oxidative and protective properties [24]. Since they may treat cardiovascular issues, vegetables rich in flavonoids were often used as functional meals [25]. As a result of the high absorption, flavonoids have been shown to consistently create pharmacologically relevant plasma concentrations in humans [26]. According to particular research, flavonoids provided cardioprotective effects against ischemia reperfusion [27].

Linoelaidic acid has anti–inflammatory, anti–arthritic, hepatoprotective, antiandrogenic, 5–alpha–reductase inhibitor, antihistaminic, anticoronary, insectifuge, hypocholesterolemic, nematicide, anti-eczemic, and antiacne properties [28]. Phytol was also reported with antioxidant and neuroprotective, anti-microbial, anti-cancer, anti–inflammatory, and anti-diuretic activities [29]. Besides, myristic acid was reported as an anti-microbial [30], anti–inflammatory [31], antioxidant [32], food additive, and flavor agent [33]. In contrast, Dandekar et al. [2] observed that the methanolic extract of *E. oxypetalum* leaves lacked peaks of octadecanoic acid, phytol, and *n*–hexadecanoic acid when extracted using the conventional extraction technique. The influence of varying pressure and temperature that led to the detection peak of area data in GC–MS analysis may be responsible for the variations in phytochemical contents. These findings encourage additional research to be conducted on extracts and the identification of specific active chemical components with medicinal effects.

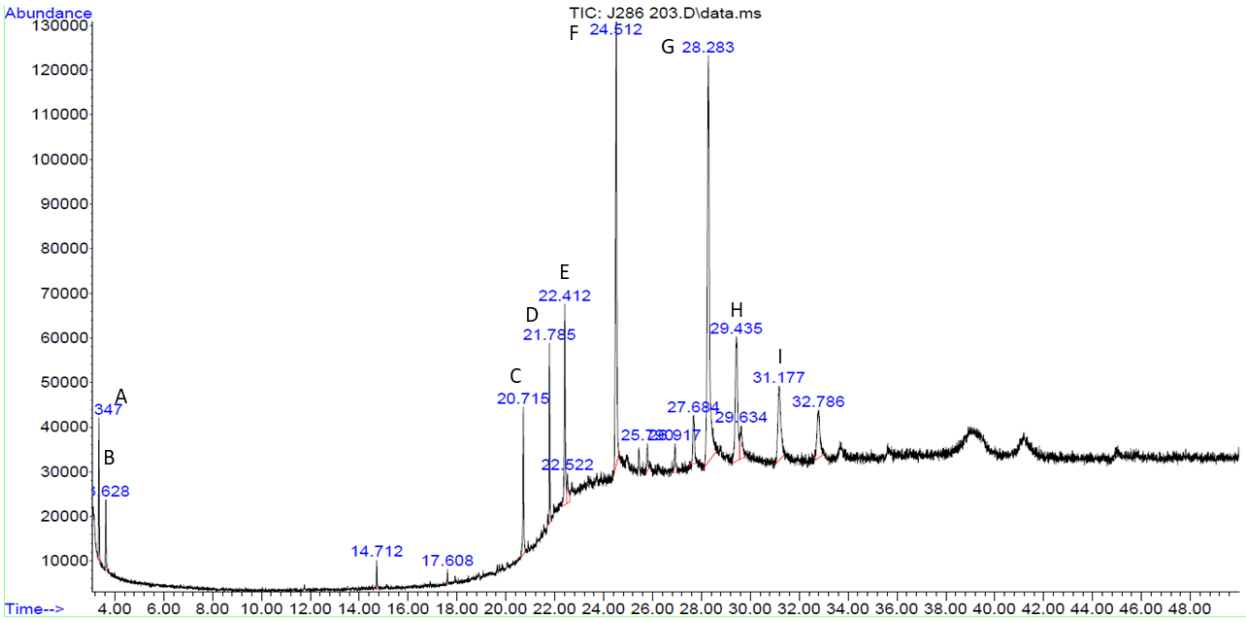
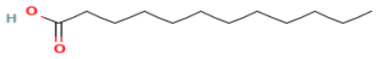
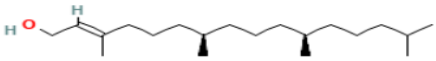
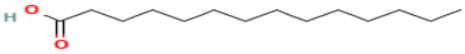
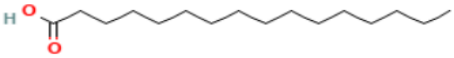
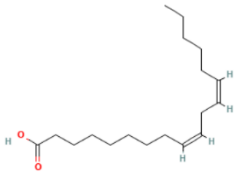
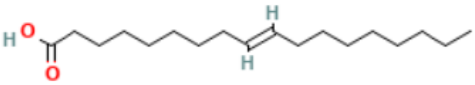


Figure 2. GC–MS chromatogram of the extract of *E. oxypetalum* leaves oil for sample 3 (30 MPa, 40 °C) based on supercritical fluid extraction with carbon dioxide extraction method (A: trichloromethane, B: toluene, C: dodecanoic acid, D: phytol, E: myristic acid, F: *n*–hexadecanoic acid, G: oleic acid, H: linoelaidic acid and I: cis, cis, cis–7,10,13–hexadecatriena)

Table 3. Phytochemical constituents compound found in the extract of *E. oxypetalum* leaves oil for sample 3 (30 MPa, 40 °C) based on supercritical fluid extraction with carbon dioxide method

Retention Time (min)	Peak area (%)	Similarity (%)	Name of Compound	Molecular formula	Molecular weight ($g \cdot mol^{-1}$)
3.3	3.1	95	Trichloromethane	CHC_{l3}	119.37
3.6	1.5	91	Toluene	C_7H_8	92.14
20.7	4.0	97	Dodecanoic acid	$C_{12}H_{24}O_2$	200.32
21.8	3.4	91	Phytol	$C_4H_8C_{l2}NOP$	187.99
22.4	6.6	99	Myristic acid	C_4H_9N	71.12
24.5	18.4	99	*n*–hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
28.3	30.7	99	Oleic acid	$C_{18}H_{34}O_2$	282.5
29.4	10.3	99	Linoelaidic acid	$C_{18}H_{32}O_2$	280.4
31.8	7.5	86	cis, cis, cis–7,10,13–hexadecatriena	$C_{17}H_{18}N_2O_4$	314.34

Table 4. The uses of fatty acids compounds found in the extract of *E. oxypetalum* leaves oil for sample 3 (30 MPa, 40 °C) based on supercritical fluid extraction with carbon dioxide method

Name of Compound	Composition (%)	Structure Of Compounds	Uses	Ref
Dodecanoic acid	3.99		anti–bacterial, anti–inflammatory, antioxidants	[21–23]
Phytol	3.41		antimicrobial, anti–inflammatory, anti–cancer, antifungal, headache, and anti–malarial	[2][29]
Myristic acid	6.56		antimicrobial, anti–inflammatory, antioxidants, food additive, and flavor agent	[30–33]
n–hexadecanoic acid	18.43		antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5–alpha reductase inhibitor, antipsychotic	[21–27]
Linoelaidic acid	10.27		anti–inflammatory, anti–arthritic, hepatoprotective, antiandrogenic, 5–alpha–reductase inhibitor, antihistaminic, anti–coronary, insectifuge, hypocholesterolemic, nematicide, anti–eczemic, and anti–acne	[2][28]
Oleic Acid	30.70		anti–obesity, lowered the risk of heart disease, reduced blood pressure, anti–cancer agent, dietary fat, cosmetics cleansing agent, antioxidants, cosmetic texture enhancer, and solubilizing agent in aerosol products	[17–20]

CONCLUSION

The results of this investigation supported the notion that environmental friendly solvents like supercritical carbon dioxide may be used to create sustainable oil products. The extract of *E. oxypetalum* leaves oil has six fatty acids compounds that possess many pharmacological properties based on the different temperatures and pressures using supercritical fluid extraction with carbon dioxide. The GC–MS analysis showed the presence of various fatty acids compounds; oleic acid, *n*–hexadecanoic acid, and linoelaidic acid. These data contributed to activities like antimicrobial, antioxidant, anti–cancer, flavoring, anti–inflammatory, and other activities. Hence, the presence of fatty acids composition is responsible for their therapeutic effects and biological activity. For future work, liquid chromatography–mass spectrometry (LC–MS) analysis would recommend to analyze the polar and non–volatile phytochemicals of the extract of *E. oxypetalum* leaves.

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