Journal of Medicinal Natural Products

Scilight

Article

Composition of *Amesiodendron chinense* (Merr.) Hu Seed Oil and Assessment of Its Nrf2/ARE Induction Activity in AREc32 Cells

Chuanchom Khuniad [1,2,*], Lutfun Nahar [3,*], Jason W. Birkett [4], Kenneth J. Ritchie [1] and Satyajit D. Sarker [1,*]

[1] Centre for Natural Products Discovery (CNPD), School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool L3 3AF, UK
[2] Department of Thai Traditional Medicine, Faculty of Health and Sports Science, Thaksin University, Phatthalung 93210, Thailand
[3] Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, The Czech Academy of Sciences, Šlechtitelů 27, 78371 Olomouc, Czech Republic
[4] Forensic Science Research Group, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool L3 3AF, UK
***** Correspondence: C.Khuniad@2020.ljmu.ac.uk (C.K.); nahar@ueb.cas.cz (L.N.) and S.Sarker@ljmu.ac.uk (S.D.S.)
Received: 7 April 2024; Revised: 26 April 2024; Accepted: 6 May 2024; Published: 14 May 2024

Abstract: Background: *Amesiodendron chinense* (Merr.) Hu (family: Sapindaceae) is a Thai medicinal plant. The seed oil of this species has been used by folk healers and local people in southern Thailand for the treatment of wounds, skin disorders and common hair problems. This study aimed at the GC-MS-based determination of the chemical composition of the seed oil of this plant, and evaluation of its Nrf2/ARE induction activity in AREc32 cells (modified human breast cancer cell line MCF-7) using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and luciferase reporter gene assays. Results: GC-MS analysis identified 9-(*E*)-octadecenoic acid (84.82%) as the main component of this seed oil. TLC-based qualitative DPPH (2,2-diphenyl-1-picrylhydrazyl) assay revealed the DPPH radical-scavenging activity of the seed oil and its chromatographic fractions. A low-level DPPH-scavenging activity was observed in the quantitative assay, but no IC_{50} value could be determined even with the highest tested concentration (10 mg/mL). Neither the oil nor its chromatographic fractions showed any significant Nrf2/ARE induction in AREc32 cells. The seed oil was noncytotoxic against the AREc32 cells. Conclusions: *A. chinense* seed oil and its fractions had a low level of free-radical scavenging property but no significant Nrf2/ARE induction activity in AREc32 cells. However, as the oil did not show any cytotoxicity at test concentrations in the MTT assay, this oil might potentially be safe to use in cosmetic formulations or as a vehicle for the dermal delivery of drug molecules.

Keywords: *Amesiodendron chinense*; Sapindaceae; seed oil; antioxidant; cytotoxicity; cancer chemoprevention; GC-MS; Nrf2/ARE induction

1. Background

Thailand is one of the most biodiversity-rich countries in Southeast Asia, which contains *ca.* 15,000 plant species, representing 8% of the world's plant species [1]. Importantly, Thai ancestors had accumulated precious experience of health care in fighting against diseases before the Sukhothai period or before 1238 CE. As a result, the unique traditional medicine system, known as "Thai Traditional Medicine" (TTM), based on Thai medicinal plants, was developed [2,3]. Although medicinal plants form the foundation of the TTM, many of these plants have never been evaluated for their phytochemical composition and bioactivities, and for most of them, their therapeutic potential is yet to be discovered.

Amesiodendron chinense (Merr.) Hu is one of the medicinal plants of the Sapindaceae family [4]. This species is a primary rainforest tree growing up to 25 m tall (Figure 1). Its Thai name is "Khun", and found in primary rainforests, native to valleys, hill forests and limestone areas, 300–1000 m altitude and growing on well-drained soil [4,5]. It is distributed from South China through Indochina to Peninsular Malaysia and Sumatra. In Thailand, it is an endemic tree species only found in Phatthalung and Trang province, the southern part [4–6]. *A. chinense*

was categorized as 'near threatened' by the International Union for Conservation of Nature (IUCN) due to over-exploitation of valuable timber in certain regions and a restricted geographical distribution of this plant [7]. However, the use of seeds to produce oil is not considered a threat to this species.

Traditional healers and local people in southern Thailand externally apply *A. chinense* seed oil to treat chronic wounds such as diabetic foot and pressure ulcers, itching, skin allergies and inflammation caused by insect bites, skin diseases such as ringworm, scabies, and athlete's foot. Moreover, it is applied as a massage oil for relieving muscle pain and a hair tonic for giving shiny-black hair and antidandruff treatment [6,8,9]. This oil has also been used as an excipient in carious cream formulations [9].

a **b** **c**

Figure 1. Tree (**a**), fruits (**b**) and seeds (**c**) of *A. chinense.*

Previous phytochemical and pharmacological studies of this species identified phenolic compounds including flavonoids amesiflavones A–C, and lignans (+)-aptosimon (+)-isolariciresinol (−)-cleomiscosin A and (−)-cleomiscosin C from the leaves. Amesiflavones A and B, (+)-aptosimon, (−)-cleomiscosins A and C, and (+)-isolariciresinol showed cytotoxic activity against human cancer cell lines including KB (a subline of the ubiquitous KERATIN-forming tumour *cell line* HeLa), SK-LU-1 (lung cancer), MCF-7 (breast cancer), HepG-2 (liver cancer), and SW-480 [4,10]. Further phenolic compounds, astragalin, (−)-catechin, (−)-*epi*-catechin, chrysoeriol, kaempferide 3-*O*-β-D-glucopyranoside and quercetin 3-*O*-β-D-glucopyranoside were isolated from the flowers [11]. Moreover, *A. chinense* seed oil was shown to contain six fatty acids including, arachidic acid, eicosenoic acid, linoleic acid, oleic acid, palmitic acid, and stearic acid [6].

Although the seed oil of *A. chinense* has been used in folk medicine in southern Thailand with effective treatment outcomes, there are limited phytochemical and pharmacological studies reported on this species to date. No published research articles on pharmacological studies of *A. chinense* seed oil have been found in the literature. Thus, this study aimed at the GC-MS analysis of the seed oil of this plant, and evaluation of its Nrf2/ARE induction activity in AREc32 cells using the MTT and luciferase reporter gene assays**.**

2. Methods

2.1. Plant Materials

Amesiodendron chinense (Merr.) Hu seed oil was supplied by Sawat Chandang, a traditional healer in Phatthalung province, Thailand. Seed oil was extracted using traditional methods as briefly described here. The fruits were sun-dried and cracked, and the peels were removed. The seeds were ground and cooked by steaming. After that, the cooked seeds were placed in a wicker container (Figure 2a) and put in wooden equipment (Figure 2b). Then logs were inserted on both sides of the equipment to compress the seeds. Lastly, seed oil was collected, filtered, and kept in a glass bottle (Figure 2c).

a **b** **c**

Figure 2. Wicker container (**a**), wooden equipment (**b**) and seed oil (**c**) of *A. chinense*.

2.2. GC-MS Analysis

The chemical constituents of the seed oil sample were analyzed on an Agilent 6890N network GC system coupled to an Agilent 5790 mass selective detector (Agilent, Santa Clara, CA, USA). Separations were conducted using an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The seed oil sample was diluted 10 times in *n*-hexane and then the sample (1 μL) was injected in the split mode with a split ratio of 1:50. The column oven temperature was programmed as follows: the beginning of 150 °C kept for 3 min, rising by 20 °C/min to 280 °C and maintaining for 4 min. The total run time was 14 min. Helium was used as a carrier gas at a flow rate of 1 mL/min. The mass-spectrometer was accomplished in the range of 41–500 *m*/*z* in the EI mode at 70 eV at an MS source temperature of 230 °C.

2.3. VLC Fractionation

Vacuum liquid chromatography (VLC) was carried out as described by Reid and Sarker (2012) [12] with suitable modifications. In brief, a portion of seed oil (10 mL) was mixed with normal silica gel (70–230 mesh) (Sigma Aldrich, St. Louis, MO, USA) and loaded on the top of a VLC column pre-packed with TLC-grade silica gel 60 H (Sigma Aldrich, USA). The column was washed with 200 mL of *n*-hexane and eluted with a stepwise mobile phase gradient, consisting of 2% (twice), 4%, 6%, 10%, 20%, 30%, 40% and 50% DCM in *n*-hexane and finally, 100% DCM (200 mL each) under vacuum. Ten different fractions (F1-F10) were collected separately, dried under a rotary evaporator, and stored in glass vials at 4°C until used.

2.4. TLC Analysis

Silica gel 60 coated with fluorescent indicator F_{254} on TLC aluminium foil (Sigma Aldrich, USA) was used as a stationary phase. Seed oil and 10 VLC fractions were re-dissolved (10 mg/mL) in DCM and spotted on the TLC plate. The TLC plate was developed using 5% of ethyl acetate in *n*-hexane as a mobile phase. After that, the plate was dried, and visualized under UV light at 254 and 365 nm in a UV cabinet (Analytik Jena, Upland, CA, USA). The developed plate was then sprayed with an anisaldehyde-sulphuric acid reagent followed by heating in an oven (Nabertherm, Lilienthal, Germany) at 110 °C for 10 min. Fractions with similar TLC fingerprints were combined.

2.5. DPPH Assay

Seed oil and its fractions were screened for free-radical scavenging properties using the DPPH assay following the method described by Takao et al. [13] with proper modifications [14,15]. DPPH powder (Sigma Aldrich, USA) (8 mg) was dissolved in isopropanol (100 mL) to obtain a concentration of 80 μg/mL.

2.5.1. Qualitative Assay

All tested solutions (10 mg/mL) including quercetin (Sigma Aldrich, USA), a positive control (1 mg/mL) were applied on the TLC plate and followed by the TLC development step as aforementioned procedure. The DPPH solution was sprayed onto the developed TLC plate and left the TLC plate for 30 min to allow the reactions to occur. The white spots against the purple background were indicative of the antioxidant potential of the samples.

2.5.2. Quantitative Assay

Ten-fold serial dilutions of each tested sample were prepared in isopropanol from the stock concentrations (10 mg/mL) to obtain concentrations of 1.0, 0.1, 0.01, 0.001, 0.0001 and 0.00001 mg/mL. After that, 2 mL of diluted solutions were mixed with 2 mL of DPPH and allowed to stand at room temperature for 30 min for any reaction to occur. Absorbance values were measured at 517 nm using a Jenway 7315 Advanced UV-VIS spectrophotometer (Bibby Scientific Ltd, Staffordshire, UK). The experiment was performed in triplicate. The same protocol was conducted for the positive control, quercetin (stock concentration 1 mg/mL). The percentage of DPPH scavenging activity at each concentration expressed as % inhibition was calculated by using the following formula:

$$\text{Inhibition (\%)} = (A_{control} - A_{sample}) \times 100/A_{control} \quad (1)$$

where $A_{control}$ was the absorbance value of the blank control (containing all reagents except for the tested sample) and A_{sample} was the absorbance value of the tested sample/positive control.

2.6. Preparation of Cell Lines and Cell Culture

AREc32 cells (modified the human breast cancer cell line MCF-7) were used for cell culture work [16]. All cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) high glucose (Biosera Europe, Paris, France) supplemented with 10% foetal bovine serum (FBS) (Thermo Fisher Scientific, USA), 5% penicillin-streptomycin suspension (Sigma Aldrich, USA) and geneticin G418 (0.8 mg/mL) (Thermo Fisher Scientific, Waltham, MA, USA) and then maintained in an incubator (Binder, Germany) at 37 °C under 5% CO_2 and 95% humidity**.** The cells were seeded into 96-well plates (Corning, USA) at a cell density of 1.2×10^4 cells in 100 µL of complete medium per well and incubated for 24 h before each experiment started. All experiments were performed in three independent experiments (5 replicates/experiment).

2.7. MTT Assay

MTT assay [17] was used to determine the cytotoxicity of the seed oil and its fractions. Cells were treated with 100 µL of the following concentrations 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1 mg/mL. Negative (untreated cells), positive (20% dimethyl sulfoxide, DMSO) and vehicle controls (0.4–1% ethanol) were also included in each experiment. After 24 h incubation, 20 µL of the MTT solution (5 mg/mL) was added to each well and incubated for 4 h. The MTT solution was discarded and 100 µL of DMSO was added in each well to dissolve the purple formazan product. The absorbance of the formazan product of viable cells was read using the microplate reader (Tecan, Männedorf, Switzerland) at 570 nm. The mean % cell viability was calculated as follows:

$$\% \text{ Cell viability} = (\text{absorbance of treated cells}/\text{absorbance of untreated cells}) \times 100 \quad (2)$$

2.8. Luciferase Assay

Luciferase assay was performed as described by Basar et al. [16]. AREc32 cells were treated with seed oil and its fractions at a non-cytotoxic concentration which was assessed by the MTT assay for 24 h. Cells were washed with 100 µL of phosphate-buffered saline (PBS), then 20 µL of 1X luciferase reporter lysis buffer (Promega, Southampton, UK) was added to each well followed by a freeze–thaw cycle (−20 °C) for 24 h to achieve complete cell lysis. The cell lysate was thawed and transferred to a white opaque 96-well plate and mixed with 100 µL of freshly prepared luciferase assay reagent. The bioluminescence was measured using the microplate reader (Tecan, Switzerland). The Luciferase activity was expressed as folds induction relative to untreated cells. Positive control of 10 µM *tert*-butylhydroquinone (*t*BHQ) was also used.

2.9. Statistical Methods

All experiments were conducted in triplicate. All results were presented as means ± standard deviation. The graphs were plotted using non-linear regression using Microsoft Excel version 2019.

3. Results

3.1. GC-MS Analysis: Composition of the Seed Oil

The seed oil derived from *A. chinense* had a yellow colour. The identified components, retention time, molecular formula, and relative peak area (%) are displayed in Table 1. The GC-MS profile is shown in Figure 3. A total of eight compounds were identified, representing 100% of the total oil. The major components of the seed oil were 9-(*E*)-octadecenoic acid (84.82%) (Figure 4) followed by di-isooctyl phthalate (8.78%), 2,3-dihydroxypropyl elaidate (1.86%) and ethyl *iso*-allocholate (1.37%).

Table 1. Chemical composition of seed oil extracted from *A. chinense*.

No	Retention Time (min)	Molecular Formula	Compounds	Relative Peak Area (%)
1	7.4	$C_{27}H_{56}$	Heptacosane	0.20
2	7.76	$C_{16}H_{32}O_2$	*n*-Hexadecanoic acid	0.47
3	8.72	$C_{18}H_{34}O_2$	9-(*E*)-Octadecenoic acid	84.82
4	10.25	$C_{21}H_{40}O_4$	2,3-Dihydroxypropyl elaidate	1.86
5	10.54	$C_{18}H_{34}O_2$	13-(*Z*)-Octadecenoic acid	0.85
6	10.62	$C_{24}H_{38}O_4$	Diisooctyl phthalate	8.78
7	10.74	$C_{18}H_{34}O_2$	Oleic acid	0.6
8	10.86	$C_{18}H_{34}O_2$	13-*(E)*-Octadecenoic acid	1.07
9	12.48	$C_{26}H_{44}O_5$	Ethyl *iso*-allocholate	1.37

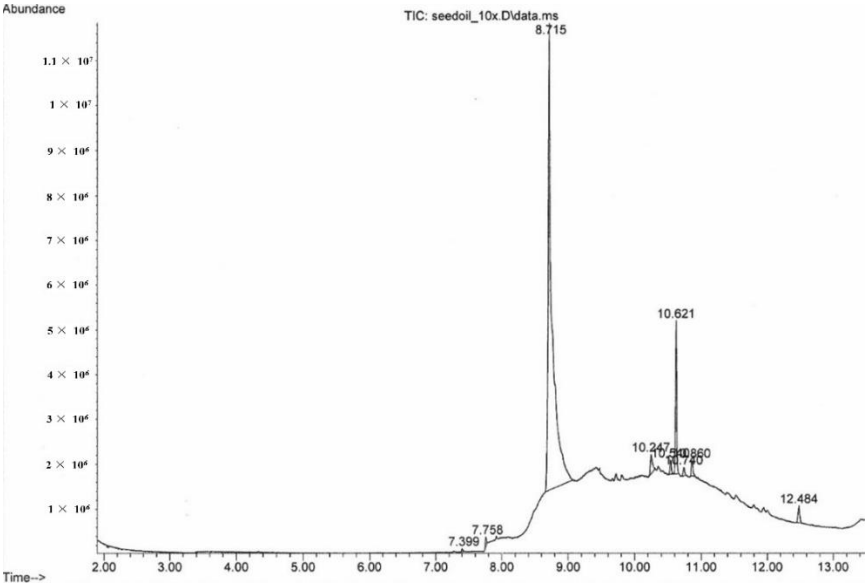


Figure 3. GC-MS chromatogram of *A. chinense* seed oil.

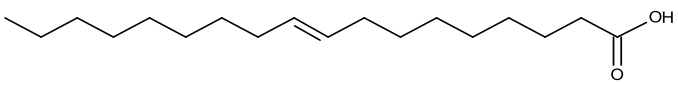
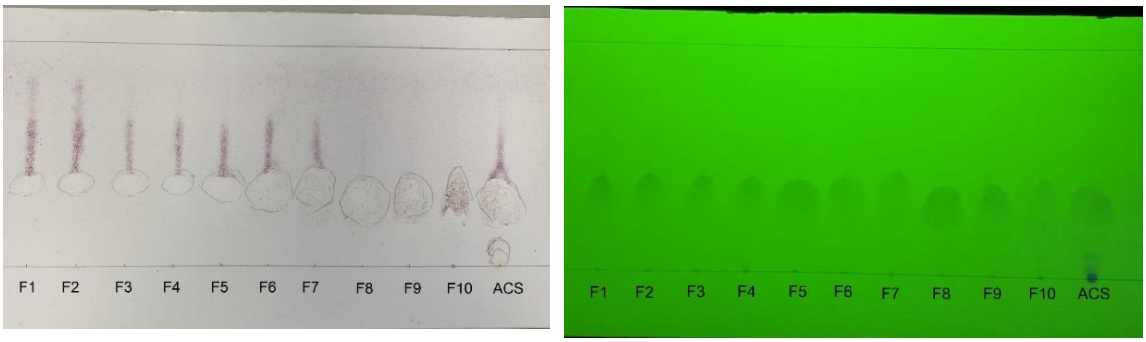


Figure 4. Chemical structure of 9-(*E*)-octadecenoic acid.

3.2. TLC Screening of the Oil and Its Chromatographic Fractions

The TLC chromatogram of *A. chinense* seed oil (ACS) and its VLC fractions (F1–F10) with different detection methods are presented in Figure 5. VLC fractions with similar TLC profiles were then combined as follows: F1 + F2, F3 + F4, F6 + F7 and F8 + F9.



A **B**

Figure 5. TLC fingerprint of *A. chinense* seed oil (ACS) and its VLC fractions with visualization under UV 254 nm (**A**) and visible light after derivatization with anisaldehyde-sulphuric acid reagent (**B**); solvent system: *n*-hexane: ethyl acetate (9.5:0.5).

3.3. DPPH-Scavenging Activity

The TLC-based qualitative DPPH assay of the *A. chinense* seed oil and its VLC fractions revealed white spots against a purple background (Figure 6), indicating the presence of compounds with free-radical scavenging activity. The seed oil itself showed a maximum of 4.51% inhibition of DPPH at its highest tested concentration of 10 mg/mL, (Table 2), and the % inhibition of the tested VLC fractions at 10 mg/mL was between 0.668% to 2.33%. At lower concentrations (0.00001–0.1 mg/mL), the seed oil and the VLC fractions F1 + 2, F3 + 4 and F5 did not show any quantifiable DPPH-scavenging activity. Among the VLC fractions, the fraction F10 showed the highest % of inhibition (2.33%) at 10 mg/mL concentration. While the 50% inhibition value (IC_{50}) for the seed oil and its VLC fractions could not be determined even at the highest tested concentration, quercetin, as a positive control, reached 50% inhibition of DPPH at a concentration between 0.01 and 0.1 mg/mL.

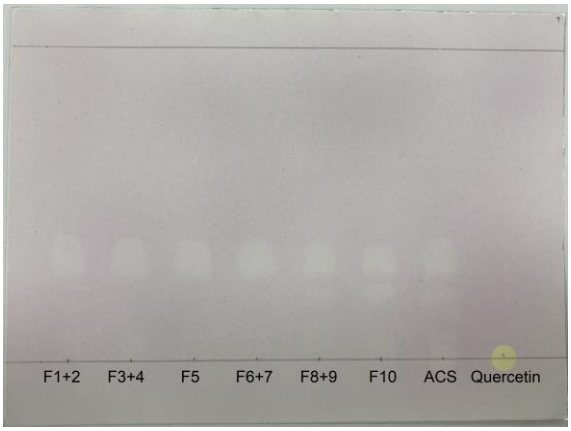


Figure 6. TLC plate of *A. chinense* seed oil (ACS) and fractions after spraying with DPPH reagent.

Table 2. Quantitative DPPH assay of the seed oil and its VLC fractions.

Conc. (mg/mL)	% Inhibition of DPPH							
	Quercetin	Seed Oil	VLC fractions					
			F1 + 2	F3 + 4	F5	F6 + 7	F8 + 9	F10
0.00001	-	-	-	-	-	0.165	-	0.359
0.0001	0.499	-	-	-	-	0.824	-	0.277
0.001	5.668	-	-	-	-	0.494	0.273	0.554
0.01	33.305	-	-	-	-	0.301	0.276	0.416
0.1	71.289	-	-	-	-	0.357	0.277	0.693
1	93.300	1.002	0.002	0.028	-	0.165	0.853	0.915
10	NP	4.514	0.668	1.951	-	0.770	1.323	2.328

- = No inhibition; NP = Not performed.

3.4. Cytotoxic Activity: MTT Assay

The seed oil and its fractions at the concentration ranging from 0.0125–1 mg/mL were assessed for cytotoxicity against AREc32 cells using the MTT assay to identify a non-cytotoxic concentration of each treatment. Ethanol was used at 0.4–1% *v*/*v* as the vehicle control which did not show toxicity. The non-cytotoxic concentrations chosen for the luciferase assay were 0.4, 1.0, 0.1, 0.4, 0.2, 0.4 and 0.8 mg/mL, respectively, for the seed oil, F1 + 2, F3 + 4, F5, F6 + 7, F8 + 9 and F10.

3.5. Chemopreventive Activity: Luciferase Assay

Non-toxic concentrations of seed oil and fractions as shown above were assessed for their chemopreventive property using the Luciferase assay by measuring induction of Nrf2 activation in AREc32 cells. As shown in Figure 7, *tert*-butylhydroquinone (tBHQ), a well-known Nrf2 activator at 10 μM was used as a positive control, produced a 23.2-fold activation of Nrf2 activity compared to the negative control. Seed oil and its fractions did not show any significant Nrf2/ARE induction (less than 2-fold compared to control).

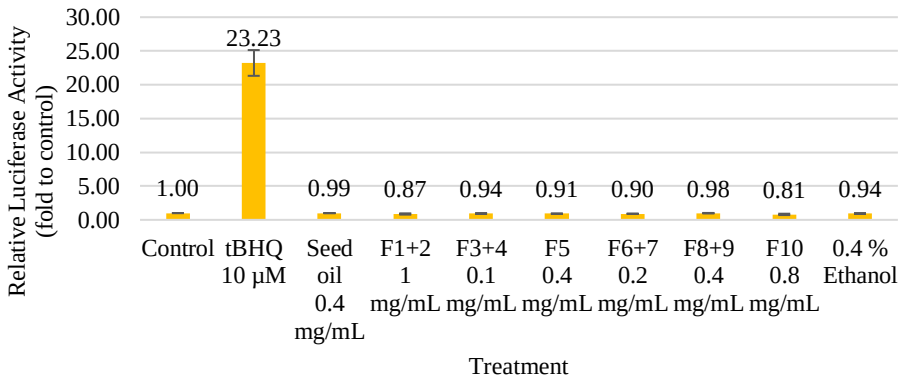


Figure 7. Luciferase activity of seed oil and its fractions on AREc32 cells.

4. Discussion

The seed oil of *A. chinense* showed the presence of long-chain alkanes and fatty acids, which is quite typical of such a fixed oil [18]. 9-(*E*)-Octadecenoic acid (84.82%), also known as elaidic acid [19], was the main component of this oil. Therefore, it is reasonable to say that the physicochemical properties of this oil are mainly because of this compound. This result is, to some extent, in agreement with an earlier report [6], where 9-(*E*)-octadecenoic acid was reported as the main component (57.14%) of *A. chinense* seed oil, but in much less proportion. However, the previous study found other fatty acids including octadecanoic acid, 9,12-octadecadienoic acid, eicosenoic acid and 11-eicosenoic acid, which were not found in the current study. This variation in the proportion of 9-(*E*)-octadecenoic acid present in the oil and the composition of the oil could be linked to the differences in the oil sample preparation for the GC-MS analysis, i.e., in the form of fatty acid methyl ester, instead of the unaltered oil itself or because of variations in geographical origin, drying method, oil extraction method and several other factors related to processing. 9-(*E*)-Octadecenoic acid, with a *trans*-configured double bond at C-9 (Figure 4), is an oleic acid *trans* isomer. It is classified as a member of the long-chain fatty acids. Consumption of this *trans* fatty acid increases low-density lipoprotein (LDL) cholesterol and decreases high-density lipoprotein (HDL) cholesterol, which is associated with an increased risk of cardiovascular disease [20,21]. However, in Thailand, the ethnobotanical uses of the seed oil of *A. chinense* are confined to external uses only, e.g., medicinal oil for chronic wounds, itching, skin allergies and inflammation and other skin conditions, and as a massage oil and hair tonic [6,8,9]. Therefore, the presence of 9-(*E*)-octadecenoic acid as the major compound in this oil will have no consequences for cardiovascular diseases.

There are many plant seed oils that possess excellent antioxidant property [22], but the seed oil of *A. chinense* did only have a low level of DPPH-scavenging activity as found in the current study. Antioxidants are well-known to be associated with the chemopreventive effect of natural products [23]. In the current study, the seed oil and its fractions did not show any significant cancer chemopreventive activity as indicated by extremely low level of induction of Nrf2/ARE activity in AREc32 cells. The absence of any significant cancer chemopreventive activity of the oil could be explained by the absence of any considerable antioxidant activity in this oil. However, it is noteworthy, that this oil or its fractions did not show any significant cytotoxicity in the MTT assay, which could provide support for its safety in external use. In cosmetic and pharmaceutical industries, it is often important to find and use excipient or vehicle that is non-toxic to use in the products only for external use [24]. To this end, the seed oil of *A. chinense*, because of its non-toxicity to cells, could be a suitable candidate for such applications.

This is the first report on the assessment of the radical-scavenging, cytotoxic and chemopreventive activities of *A. chinense* seed oil, and GC-MS analysis revealing the composition of this fixed oil.

5. Conclusion

The present study has demonstrated that the major components of the seed oil of *A. chinense* are long-chain alkenes and fatty acids. Seed oil and its chromatographic fractions did not display any noticeable cytotoxicity against the AREc32 cells at the tested concentrations. They exhibited a low level of DPPH-scavenging activity and chemopreventive potential at tested concentrations. The absence of any significant cytotoxicity in the oil might be considered a positive outcome regarding its potential use as a vehicle in cosmetic products (e.g., an excipient in carious cream formulations) or in pharmaceutical medicinal products for external use as well as its traditional use as a massage oil.

Abbreviations:

ARE	Antioxidant Responsive Element
DPPH	2,2-Diphenyl-1-picrylhydrazyl
GC-MS	Gas Chromatography-Mass Spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Nrf2	*Nuclear factor erythroid 2-related factor 2*
tBHQ	*tert*-Butylhydroquinone
TLC	Thin Layer Chromatography
VLC	Vacuum Liquid Chromatography

Author Contributions: C.K., L.N., K.J.R. and S.D.S. contributed to conceptualization and designing of this project. J.W.B. designed the GC-MS work and analysed the GC-MS data. L.N., K.J.R. and S.D.S. supervised the work, while C.K. conducted the main laboratory work. All authors contributed to writing of this manuscript. L.N., K.J.R. and S.D.S. performed editing of the manuscript.

Funding: The European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and the Czech Agency Grants - Project 23-05474S and Project 23-05389S to Lutfun Nahar, and the Royal Thai Government PhD Studentship to Chuanchom Khuniad.

Institutional Review Board Statement: No humans or animals have been used in this study.

Informed Consent Statement: Not applicable

Data Availability Statement: All data can be accessed by requesting the corresponding author.

Acknowledgments: Chuanchom Khuniad gratefully acknowledges the Royal Thai Government for the financial support throughout her study at Liverpool John Moores University and thanks Sawat Chandang for providing *A. chinense* seed oil. Lutfun Nahar gratefully acknowledges the financial support of the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and the Czech Agency Grant - Project 23-05474S and Project 23-05389S.

Conflicts of Interest: The authors declare that they do not have any competing interests.

References

1. Convention on Biological Diversity, Thailand - Main Details Biodiversity Facts. Available online: https://www.cbd.int/countries/profile/?country=th (accessed on 2 July 2023).
2. Chokevivat, V.; Chuthaputti, A. The role of Thai traditional medicine in health promotion. Proceedings of the 6th Global Conference on Health Promotion. Bangkok, Thailand, on 7-11 August 2005. Citeseer: Princeton, NJ, USA, 2005, 1–25.
3. Kanjanahattakij, N.; Kwankhao, P.; Vathesatogkit, P.; et al. Herbal or traditional medicine consumption in a Thai worker population: pattern of use and therapeutic control in chronic diseases. *BMC Complement Altern. Med.* **2019**, *19*, 258.
4. Ban, H.V.; Van, T.T.T.; Chien, V.V.; et al. Flavone C-glycosides from the leaves of *Amesiodendron chinense*. *Phytochem. Lett.* **2020**, *40*, 105–108.
5. National Parks Board of Singapore, *Amesiodendron chinense* (Merrill) Hu. Available online: https://www.nparks.gov.sg/FloraFaunaWeb/Flora/2/7/2707 (accessed on 2 July 2023).
6. Sampantamit, T.; Katemai, W. Utilization and chemical composition of Khun oil in tambon Klongchalern and tambon Charad, Knog-hra district, Phatthalung province. Available online: http://kb.tsu.ac.th/jspui/bitstream/123456789/1924/1/%E0%B8%97%E0%B8%B4%E0%B8%9E%E0%B8%A2%E0%B9%8C%E0%B8%97%E0%B8%B4%E0%B8%A7%E0%B8%B2%20%E0%B8%AA%E0%B8%B1%E0%B8%A1%E0%B8%9E%E0%B8%B1%E0%B8%99%E0%B8%98%E0%B8%A1%E0%B8%B4%E0%B8%95%E0%B8%A3%2000153185.pdf (accessed on 7 April 2024)
7. World Conservation Monitoring Centre, *Amesiodendron chinense*. The IUCN Red List of Threatened Species 1998. Available online: https://www.iucnredlist.org/species/35893/9960183 (accessed on 2 July 2023).
8. Upho, U. Ethnobotany of Buddhist and Muslim Thais in some locations in the lower part of southern Thailand. PhD thesis, Chiangmai University, Chiangmai, Thailand, 30 September 2005.
9. Wangpradit, N.; Macha, S.; Phooteh, N.; et al. Determination of required hydrophilic-lipophilic balance of *Amesiodendron chinense* (Merr.) Hu oil and development of stable cream formulation. *Lipids Cosmet.* **2022**, *29*, 2022011.
10. Ban, H.V.; Van, T.T.T.; Chien, V.V.; et al. Lignans from leaves of *Amesiodendron chinense* and their cytotoxic activity. *Vietnam J. Sci. Technol.* **2020**, *58*, 442–449.
11. Ban, H.V.; Van, T.T.T.; Chien, V.V.; et al. Flavonoids from flowers of *Amesiodendron chinense*. *Vietnam J. Sci. Technol.* **2020**, *58*, 676–684.
12. Reid, R.G.; Sarker, S.D. Isolation of natural products by low-pressure column chromatography. In Natural Products Isolation, 3rd ed.; Sarker, S. D. and Nahar, L. (ed.); Humana Press: Totowa, NJ, USA, 2012, pp. 155–188.
13. Takao, T.; Kitatani, F.; Watanabe, N.; et al. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1780–1783.
14. Chima, N.K.; Nahar, L.; Majinda, R.R.T.; et al. Assessment of free-radical scavenging activity of *Gypsophila pilulifera*: assay-guided isolation of verbascoside as the main active component. *Rev. Bras. Farmacogn.* **2014**, *24*, 38–43.
15. Kumarasamy, Y.; Byres, M.; Cox, P.J.; et al Screening seeds of some Scottish plants for free radical scavenging activity. *Phytother. Res.* **2007**, *21*, 615–621.
16. Basar, N.; Nahar, L.; Oridupa, O.A.; et al. Utilization of the ability to induce activation of the nuclear factor (erythroid-derived 2)-like factor 2 (Nrf2) to assess potential cancer chemopreventive activity of liquorice samples. *Phytochem. Anal.* **2016**, *27*, 233–238.
17. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
18. Uddin, G.; Rauf, A.; Gull, S.; et al. Proximate chemical composition and biological profile of fatty acids of *Withania somnifera* L dunal. *J. Med. Plants Res.* **2013**, *27*, 2034−2039.

19. Low, J.N.; Scrimgeour, C.; Horton, P. Elaidic acid (*trans*-9-octadecenoic acid). *Crystallogr. Commun.* **2005**, *61*, o3730−o3732.
20. Nielsen, L.V.; Krogager, T.P.; Young, C.; et al. Effects of elaidic acid on lipid metabolism in HepG2 cells, investigated by an integrated approach of lipidomics, transcriptomics and proteomics. *PloS one* **2013**, *8*, e74283.
21. Abbey, M.; Nestel, P.J. Plasma cholesteryl ester transfer protein activity is increased when *trans*-elaidic acid is substituted for cis-oleic acid in the diet. *Atherosclerosis* **1994**, *106*, 99−107.
22. Frantianni, F.; d'Acierno, A.; Ombra, M.N.; et al. Fatty acid composition, antioxidant and *in vitro* anti-inflammatory activity of five cold-pressed *Prunus* seed oils and their anti-biofilm effect against pathogenic bacteria. *Front. Nutr*. **2021**, *8*, 775751.
23. Ranjan, A.; Ramachandran, S.; Gupta, N.; et al. Role of phytochemicals in cancer prevention. *Int. J. Mol. Sci.* **2019**, *20*, 4981.
24. Alvarez, A.M.; Rodriguez, M.L.G. Lipids in pharmaceutical and cosmetic preparations. *Grasas Aceites* **2000**, *52*, 74–96.