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Physiochemical properties, antibacterial and antioxidant activities of *Terminalia catappa* seed oils from two extracting processes

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Abstract

Terminalia catappa is a widespread medium tree species in many tropical countries. While the majority of the studies up to date focuses on the aerial part of the plant such as leaf, stem bark and fruit, information about the phytochemical property as well as the biological property of the edible seed is still scarce. This study was the first to explore the fatty acid composition, antibacterial and antioxidant activities of the seed oil from *T. catappa* grown in Vietnam. The results showed that both the hot-pressed and cold-pressed oils contained a high level of unsaturated fatty acids such as oleic (~32%) and linoleic acids (28.38%-29.2%), as well as saturated fatty acids such as palmitic acid (~33.3%-33.61%). The presence of eicosadienoic acid in *T. catappa* seed oils was reported in this study for the first time. These oils displayed antibacterial activity against 5 out of 12 tested strains such as *Bacillus cereus*, *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus.* The antioxidant activity of the oils was also recorded by DPPH radical scavenging assays with IC_{50} values of 950 µg/ml and 2529 µg/ml for cold-pressed oil and hot-pressed oil respectively. This study has provided promising extracting methods and resulted in oils that could be good candidates for developing food sources with valuable fatty acids, antioxidant and antibacterial capacities against both Gram-positive and negative bacteria in the human diet.

Keywords

Terminalia catappa, seed oil, fatty acid composition, antibacterial activity, antioxidant activity

Introduction

Terminalia catappa Linn. (Combretaceae) or tropical almond is a widely spread monoecious tree growing in tropical regions throughout the world, especially in coastal areas of South and Southeast Asia, Papua New Guinea, northern Australia and Africa (1, 2). The tree is mostly planted for decoration, shades, edible fruits and seeds. (1, 3). Nutritional value of the fruit peel and pulp can be vary depending on growth region and varieties with moisture (16.54%), ashes (4.11%), proteins (2.54%), lipids (14.95%), carbohydrate (11.27%), starch (19.57%), total fibers (31.68%), high amount of β-carotene, ascorbic acid, vitamin E and several mineral such as Calcium, Sodium, Potassium, Magnesium, Zinc and Iron (4, 5). The seed of *T. catappa* also has been found to have adequate nutritional value with moisture

(6.23%), ash (3.78%), lipid (54.68%), total fibers (9.97%), carbohydrate (7.68%), starch (1.22%), protein (17.66%) including all nine essential amino acids (6, 7). *T. catappa* was also used as traditional medicine to treat urinary tract infection (8), skin affections (9), liver disease, dysentery and diarrhea (10). To evaluate the medicinal potentials of this species, the majority of studies have focused on physiochemical and biological characteristics of the leaf. *T. catappa* leaf have been shown for its high potential in treatment diabetes using aqueous extract (11, 12), protection of human skin fibroblasts from oxidative stress using methanol extract (13), inhibition of metastasis of oral, lung and brain cancer (14-16). Both aqueous extract and ethanol of *T. catappa* leaf exhibited antioxidant activity, probably due to significant amount of polyphenolic and flavonoid contents (17-20). In addition, antibacterial properties of the leaf have been addressed as the aqueous leaf extract showed a wide range of antibacterial effects against *Streptococcus faecalis*, *Pseudomonas pseudoalcaligenes* (21), *Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas* spp. (22), *Helicobacter pylori* (23) and colibactin toxin-producing *Escherichia coli* (24). The ethanol extract well-inhibited *Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (25), while the methanol extract also showed a high inhibition effect against *Alcaligenes faecalis*, *Salmonella typhimurium*, *Pseudomonas* spp. and *Proteus* spp. (21). Compared to the leaf, only a few studies to date have addressed the physiochemical and biological characteristics the seed. It was shown that oil from *T. catappa* seed could be extracted in high yield (49% in mass) by n-hexane and was considered to be a new source of separate or mixture of biodiesel (3). The seed oil obtained using Soxhlet and maceration extraction methods contained a wide range of both good quality saturated (40%) and unsaturated fatty acids (60%), making it a highly putative dietary lipid (26). *T. catappa* seed oil obtained from n-hexane extraction from supercritical CO_2 extraction contained a majority of palmitic, oleic, and linoleic acids (26-28, 40). The n-hexane extracted oil also displayed a low capacity of neutralizing the DPPH free radical (28). As cold-press and hot-press are the two simple, low cost and popular extraction methods that can affect the oil quality and content (29, 30), this study aimed to determine the physiochemical properties of *T. catappa* seed oils extracted at hot and cold temperatures as well as evaluate their antioxidant and antibacterial characteristics, which has not yet been explored.

Materials and Methods

Plants

Terminalia catappa fruits were harvested from 7 locations in An Tinh ward, Trang Bang district, Tay Ninh province, Vietnam (Fig. 1). The scientific name of this species was identified by Southern Institute of Ecology, Vietnam Academy of Science and Technology.

No	Sampling Location
1	11^0 02’ 12.79”N 106^0 39’ 66.11” E
2	11^0 02’ 08.47”N 106^0 39’ 72.98” E
3	11^0 02’ 06.47”N 106^0 39’ 77.17” E
4	11^0 02’ 03.31”N 106^0 39’ 83.28” E
5	11^0 01’ 98.26”N 106^0 39’ 94.53” E
6	11^0 01’ 93.74”N 106^0 39’ 95.61” E
7	11^0 01’ 99.42”N 106^0 39’ 83.38” E

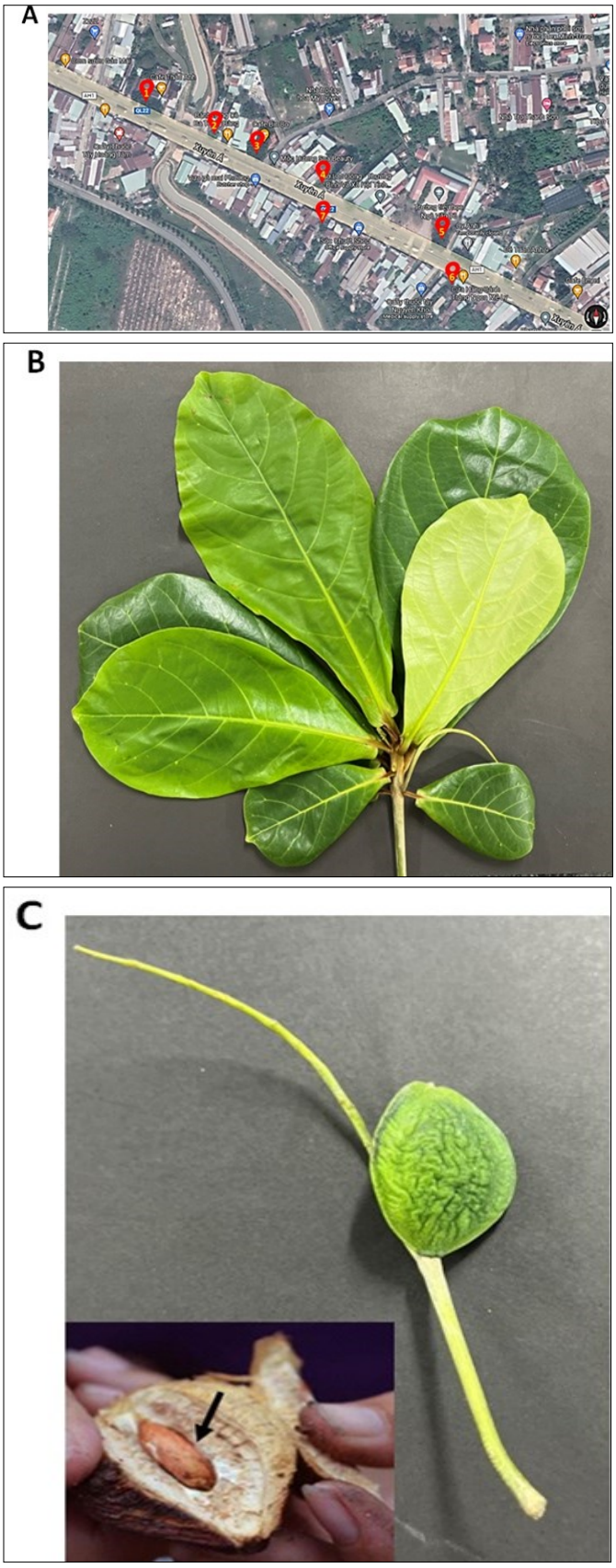


Fig. 1. *Terminalia catappa* (**A**) Collection area and sampling locations, (**B**) leaves, (**C**) fruit with nut (arrow).

Microorganisms

Twelve studied bacteria from American Type Culture Collection (ATCC) including 3 Gram-positive bacteria such as *Bacillus cereus* (ATCC 11774), *Staphylococcus aureus* (ATCC

29213), *Staphylococcus saprophyticus* (ATCC BAA-750), and nine Gram-negative bacteria such as *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883), *Salmonella enteritidis* (ATCC 13076), *Salmonella typhimurium* (ATCC 13311), *Vibrio parahaemolyticus* (ATCC 17802), *Shigella sonnei* (ATCC 25931) and *Shigella flexneri* (ATCC 12022) were used to determine the antibacterial activity of *T. catappa seed* oil.

Extraction procedures

The harvested *T. catappa* fruits were incubated at 35-38 °C for 48 hr and immersed in sodium chloride (NaCl) solution (6%, w/v) for 4 hr. Then, they were crushed to split the pulp. The seeds were collected, coat removed by hydraulic compression method, and conventionally dried at 55-60 °C for 5-6 hr until the moisture reaches 6% (31). *Terminalia catappa* seed oils were collected by screw extruder at a hot temperature (~165 °C) or low temperature (~ 42 °C) and subsequently filtered by 0.45 mm thickness polypropylene cloth to remove wax. The resulting products were called hot pressed oil (HO) or cold pressed oil (CO) and stored at 4 °C in sealed brown bottles.

Antibacterial assay

The antibacterial assay of *T. catappa* seed oil was carried out by the agar well diffusion method following the Clinical and Laboratory Standards Institute guideline (CLSI, 2010) (32). Bacterial strains were cultured in Luria-Bertani broth until their turbidity was equivalent to 0.5 McFarland, spread on the Mueller Hinton agar plates, and a 6 mm diameter hole was made using a sterile cork borer. Twenty microliters of *T. catappa* oils in dimethyl sulfoxide (DMSO) 30% were introduced into the well and the plates were incubated at 37 °C for 16-18 hrs. The antibacterial activity of the oils against tested strains was determined by the appearance of the growth inhibition zone. The positive control was conducted with Gentamycin antibiotic disc (Nam Khoa Biotek, Vietnam) and the negative control was 20 µl of DMSO 30%.

In addition, the inhibition of *T. catappa* seed oils on the growth of tested bacteria was examined by adding 10% pure oils into the Mueller Hinton culture media. The bacterial culture was incubated at 37 °C and 150 rpm. Bacterial density will be evaluated by measuring the absorbance at a wavelength of 700 nm every two hours for 12-14 hrs. The growth curves were built and calculated with Prism 8.0.2 software (GraphPad Software Inc., San Diego, California, USA).

Analysis of fatty acid composition

The composition of fatty acid in oil content was determined by Shimadzu GC-2010 gas chromatography (GC) (Shimadzu Corporations, Kyoto, Japan) equipped with a flame ionization detector using the official method of Association of Official Analytical Chemists (AOAC 2016, 996.06) (33). Free fatty acid content as oleic acid (m/m) was determined using the official method of American Oil Chemists' Society (AOCS 2009, Ca 5a-40) (34).

Reducing power assay

The Fe^{3+} reducing power of *T. catappa* seed oils was assessed at different concentrations following the method of Yen and Chen with slight modification (35, 36). The seed oils (0.5 ml) of various concentrations was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium hexacyanoferrate (2.5 ml, 1% w/v). The mixture was incubated for 20 min at 50 °C. Trichloroacetic acid solution (2.5 ml, 10% v/v) was then added to stop the reaction, followed by 10 min of centrifugation at 3000 rpm. The obtained supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride solution (0.5 ml, 0.1%, w/v). The absorbance of reaction mixture was taken at 700 nm using a GENESYS 20 spectrophotometer (Thermo Scientific, USA). Higher absorbance value indicates greater reducing power. Ascorbic acid was used as a positive control and the experiment was repeated three times and the obtained results were performed as mean ± standard deviation (SD).

Determination of antioxidant activity by DPPH radical scavenging assay

The free radical scavenging ability of *T. catappa* seed oils were determined according to the standard method (37). Seed oils (2.0 ml) in different concentrations (0–1000 µg/ml) were well-mixed with 2.0 mL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol 95% followed by 30 min incubation in the dark, and the absorbance was measured at 517 nm using GENESYS 20 spectrophotometer (Thermo Scientific, USA). A mixture of the same amount of oil samples and 2.0 ml of methanol 95% was used as the control. The experiment was repeated three times and the obtained results were performed as mean ± standard deviation (SD).

Percentage DPPH radical scavenging activity was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100\%$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then, the % of inhibition was plotted against concentration and from the graph, IC_{50} was calculated.

Results and Discussion

Fatty acid composition of T. catappa seed oils

A total of 11 fatty acids were determined in the profile of *T. catappa* seed oils (Table 1, Fig. 2). The data showed that 38.58% of the hot-pressed oil (HO) is composed of saturated fatty acids, and 61.42% of unsaturated fatty acids with 1 or 2 double bonds. Cold-pressed oil (CO) contained 39.07% and 60.93% of saturated fatty acids and unsaturated fatty acids respectively. These results were in agreement with studies in which the oil was extracted by n-hexane (3, 28, 38). Palmitic acid (C16:0) was the type of fatty acid that was found in these oils of *T. catappa* seed in the highest concentration, ranging from 33.3% to 33.61%. The unsaturated fatty acid with one double bond (oleic acid C18:1) presented in 31.8% and 31.99% while the one with 2 double bonds (linoleic acid C18:2) presented in 29.2% and

Table 1. Fatty acid composition of *T. catappa seed* oils

Characteristic	Test result (%)	
	Hot-pressed oil	Cold-pressed oil
Myristic acid (C14:0)	0.06	0.06
Palmitic acid (C16:0)	33.30	33.61
Palmitoleic acid (C16:1)	0.28	0.34
Stearic acid (C18:0)	4.56	4.72
Oleic acid (C18:1)	31.8	31.99
Linoleic acid (C18:2)	29.2	28.38
Arachidic acid (C20:0)	0.50	0.46
Linolenic acid (C18:3)	0.06	0.09
cis-11-Eicosenoic acid (C20:1)	0.08	0.08
cis-11,14-Eicosadienoic acid (C20:2)	0.06	0.05
Behenic acid (C22:0)	0.10	0.22
Free fatty acid content as oleic acid (m/m)	1.76	0.36

28.38% of the hot-pressed oil and cold-pressed oil respectively. The presence of these most abundant fatty acids was also similar to the earlier studies (3, 28). Among the minor fractions, linolenic acid (0.06% in HO-0.09% in CO) was not discovered (3). Eicosadienoic acid (C20:2, cis-11,14) is renowned for its potential to influence polyunsaturated fatty acid metabolism and change inflammatory response by macrophages (39). It is noteworthy to note that, this fatty acid, which has not been detected in several previous studies (3, 28, 38, 40), was the first to be found in both HO and CO.

Antibacterial activity

Antibacterial activity of *T. catappa* seed oils was checked on 12 bacterial strains by agar well diffusion assay (Table 2, Fig. 3). The results showed that both HO and CO displayed inhibitory effect against 5 of the 12 studied strains of both Gram-positive and Gram-negative bacteria including *B. cereus*, *S. aureus, E. coli*, *P. aeruginosa* and *V. parahaemolyticus*. A small inhibition zone of of 7.2-10.5 mm could be partially explained by the limited diffusion of oil in the agar plate (Fig. 3).

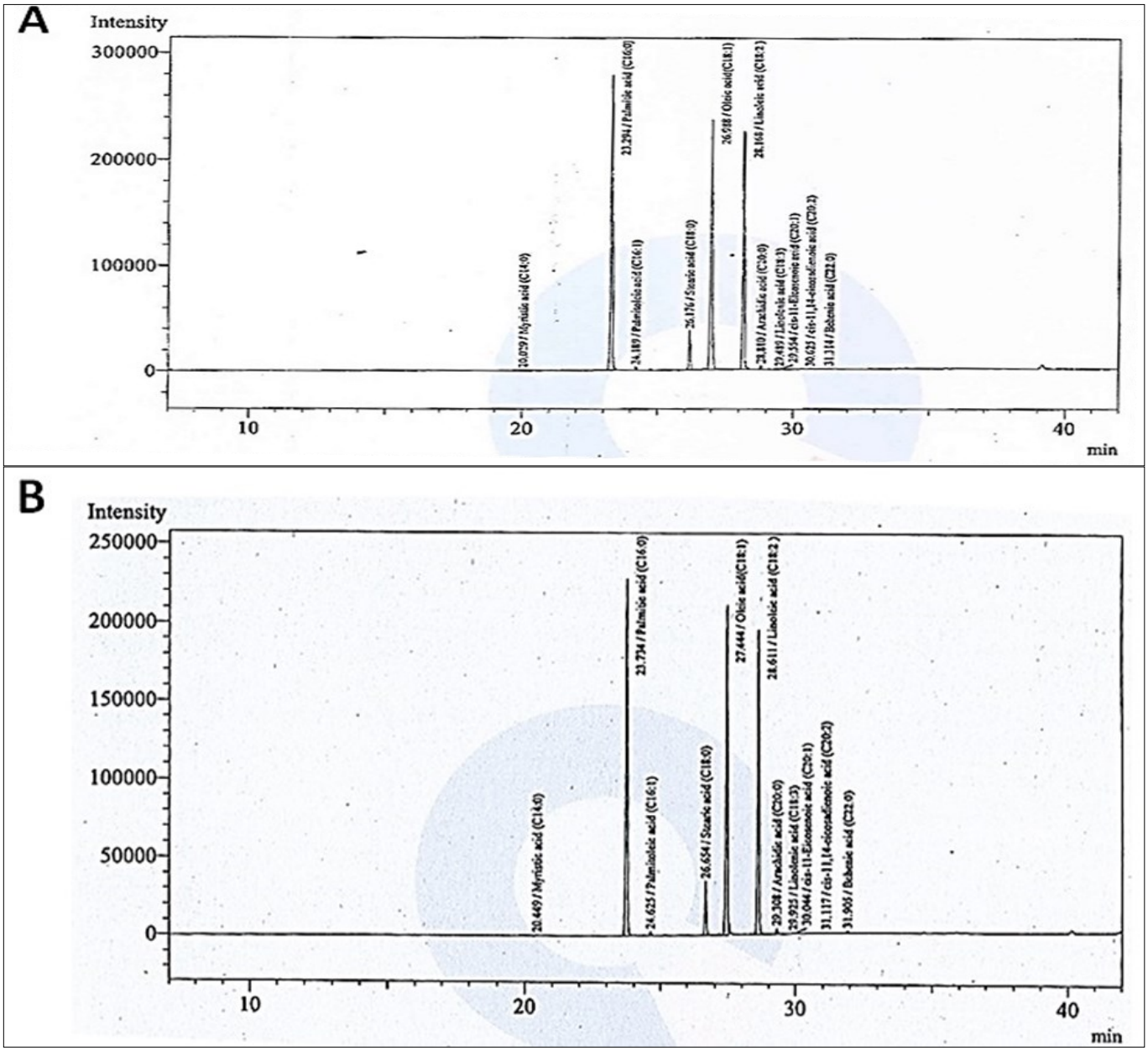


Fig. 2. Gas chromatogram of *T. catappa seed* oils. (**A**) hot-pressed oil, (**B**) cold-pressed oil.

Table 2. Antibacterial activity of *T. catappa seed* oils

No.	Tested bacteria	ATCC	Inhibition zone (mm)	
			Hot pressed oil	Cold pressed oil
1	*B. cereus*	ATCC 11778	9.2 ± 0.2	9.2 ± 0.3
2	*S. aureus*	ATCC 29213	7.2 ± 0.3	9.3 ± 0.1
3	*S. saprophyticus*	ATCC BAA750	-	-
4	*E. coli*	ATCC 25922	9.5 ± 0.6	9.0 ± 0.1
5	*E. faecalis*	ATCC 29212	-	-
6	*P. aeruginosa*	ATCC 27853	10.5 ± 0.6	7.7 ± 1.3
7	*K. pneumoniae*	ATCC 13883	-	-
8	*S. enteritidis*	ATCC 13076	-	-
9	*S. typhimurium*	ATCC 13311	-	-
10	*V. parahaemolyticus*	ATCC 17802	10.3 ± 0.3	10.1 ± 0.6
11	*S. sonnei*	ATCC 25931	-	-
12	*S. flexneri*	ATCC 12022	-	-

oils has been observed in *S. typhimurium* culture which was used as negative control (Fig. 4). The supplement of *T. catappa* seed oils has delayed the lag phase of *P. aeruginosa*, *V. parahaemolyticus*, *S. aureus* but shortened it in the case of *E. coli*. Moreover, the oils caused *B. cereus* and *E. coli* cultures to enter a premature stationary phase. No difference in the effect between the HO and CO as they reduced the *B. cereus* stationary phase yield to 53% after only 6 hrs of incubation. In the case of *E. coli* and *S. aureus*, the inhibition by CO was higher than HO, while the HO was more effective than CO in the case of *P. aeruginosa* and *V. parahaemolyticus* (Fig. 4). The antibacterial activity of *T. catappa* seed oils could be explained by the composition of the main fraction in which palmitic, oleic and linoleic acid that represented 93.98%-94.3% of total identified fatty acids in CO and HO respectively (Table 1) (41-45). It was shown that both linoleic and oleic acids isolated from *Helichrysum pedunculatum* leaves had an antibacterial effect against *B. subtilis*, *Micrococcus kristinae* and *S. aureus* (47). Additionally, linoleic acid, but not oleic, could also

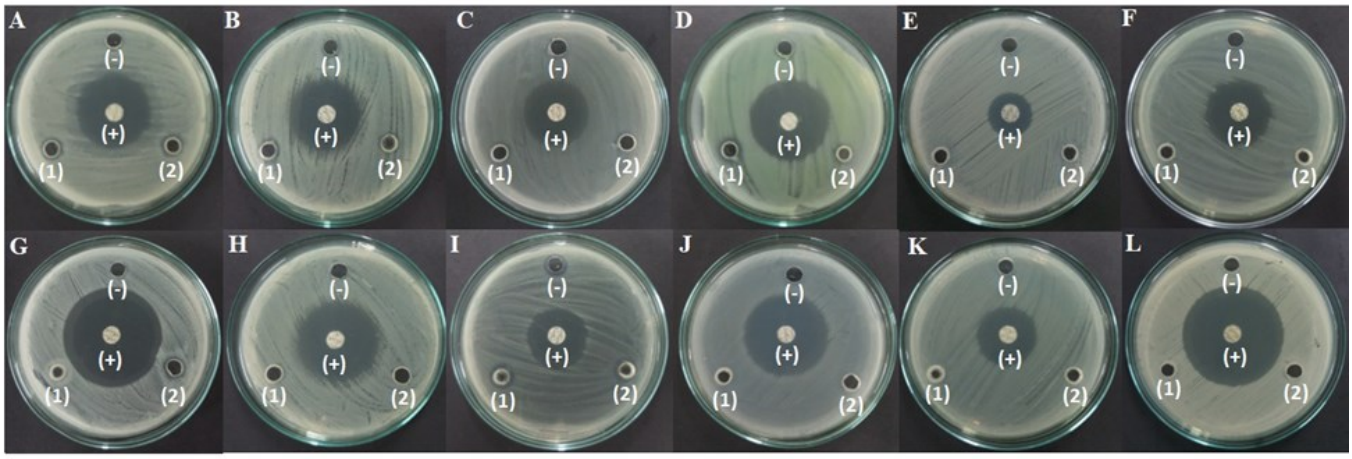
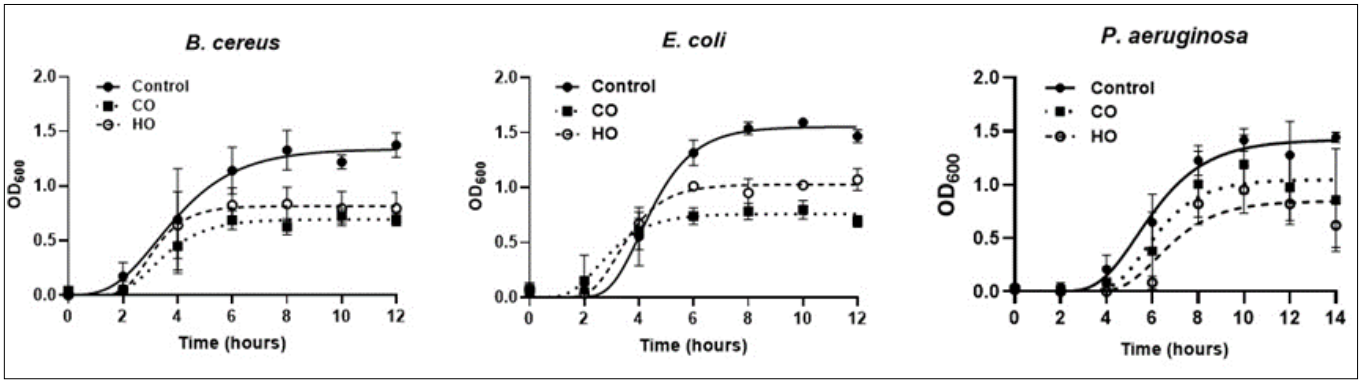


Fig. 3. Antibacterial activity of *T. catappa seed* oils. (**A**) *B. cereus,* (**B**) *E. coli,* (**C**) *E. faecalis,* (**D**) *P. aeruginosa,* (**E**) *K. pneumoniae,* (**F**) *S. enteritidis,* (**G**) *S. aureus,* (**H**) *S. typhimurium,* (**I**) *V. parahaemolyticus,* (**J**) *S. sonnei,* (**K**) *S. flexneri,* (**L**) *S. saprophyticus.* (**-**) Negative control, (**+**) Positive control, (**1**) HO, (**2**) CO.

To better determine the antibacterial effect, HO and CO were supplemented into the broth culture of the susceptible strains upon which the growth curves were generated (Fig. 4).

Differences in the 5 strains' growth curves in media with or without *T. catappa* seed oils have confirmed that these oils had antibacterial properties. The value of optical density at wavelength of 600 nm (OD_{600nm}) in the stationary phase (after 6-12 hrs of incubation) was reduced from 25% to 60% compared to the control in the cases where the HO or CO was added to the bacterial culture. No effect of the inhibit *B. cereus* and *B. pumilus* (42). Reports are on the linoleic acid from *Schotia brachypetala* leaves was effective in suppressing the growth of *B. subtilis*, *S. aureus*, *E. coli* and *K. pneumoniae* (43). In addition, palmitic acid was identified in the ethyl acetate root extract from *Pentanisia prunelloides* as a major antibacterial agent against *B. subtilis*, *S. aureus*, *E. coli* and *K. pneumoniae* (46). Palmitic and stearic acids were also reported to have a bactericidal effect against *S. aureus* and *P. aeruginosa* (47). It is worth noting that stearic acid was found to be the fourth major compound in the *T. catappa* seed oils (4.5% in HO and



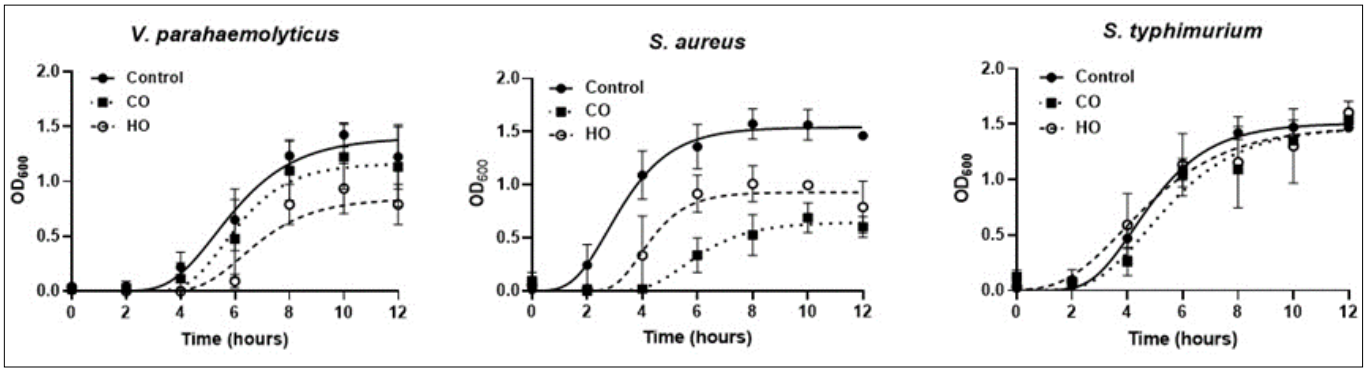


Fig. 4. Effect of *T. catappa seed* oils on bacterial growth. (**HO**) hot-pressed oil, (**CO**) cold-pressed oil Results expressed as mean±SD of triple readings against each concentration.

4.72% in CO, Table 1). Therefore, these major fatty acids such as palmitic, oleic, linoleic and stearic in the seed oils could be accounted for the main antibacterial activity against both tested Gram-positive and Gram-negative strains.

Reducing power

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. In this assay, the ability of *T. catappa seed* oils to reduce the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}) was determined. The results showed that the reducing activities of the two *T. catappa seed* oils are proportional to their concentration in the reaction mixtures and the absorbance value at 700 nm. The reducing power of HO was shown to be higher than that of CO but this difference was not statistically significant ($P > 0.05$). In addition, the reducing power of ascorbic acid was found to be many times higher than that of HO and CO. At 1000 mg/ml, the reducing power of both *T. catappa* seed oils were approximately 0.06, while the reducing power of ascorbic acid was approximately 1.7 at 100 mg/ml, suggesting that these two types of oil possessed an ability of electron donation even though it was not remarkable. In order to further demonstrate the antioxidant activity of these oils, the DPPH radical scavenging activity was determined.(Fig 5)

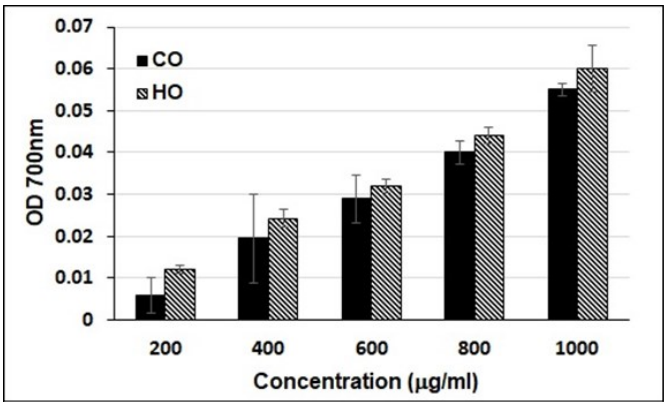


Fig. 5. Reducing power of *T. catappa seed* oils in various concentrations of oils. Results expressed as mean±SD of triple readings against each concentration.

Free radical scavenging activity

The free radical scavenging activity of *T. catappa seed* oils displayed by the DPPH radical scavenging method. The increase in oil concentration was directly proportional to radical scavenging activity (Fig. 6A). IC_{50} of HO and CO was also calculated. IC_{50} value of HO was 950 µg/mL, and 2529 µg/mL in the case of CO, which meant that the antioxidant activity of HO was about 2.5 folds higher than that of CO (Fig. 6B).

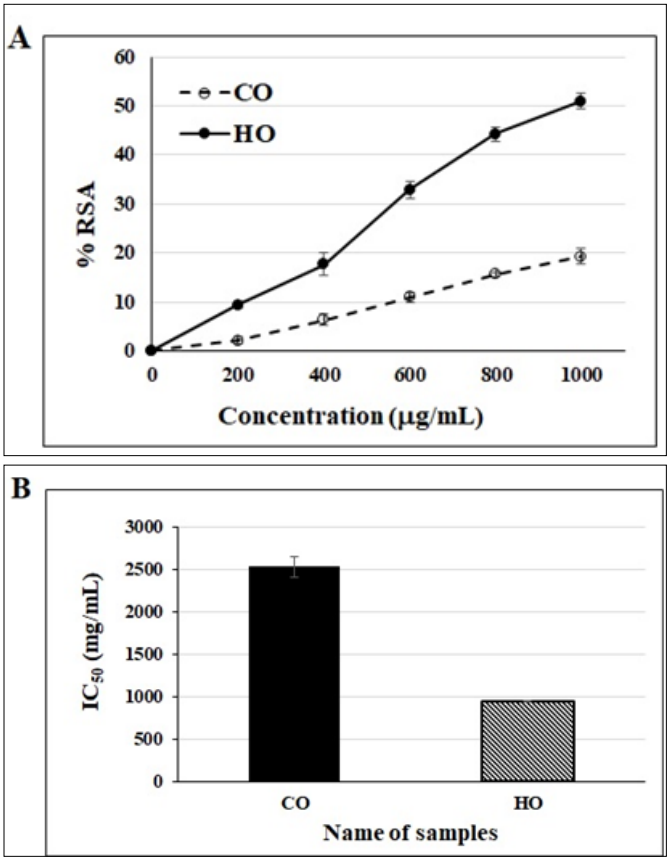


Fig. 6. Determination of a DPPH radical scavenging activity (% RSA) in various concentrations of oils (A) and IC_{50} of radical scavenging activity (B). Results expressed as mean±SD of triple readings against each concentration.

One of the reasons could be because fatty acids were better extracted at hot temperature and the free fatty acid content as oleic acid in HO was found to be 5 folds higher than in CO ($P < 0.05$) (Table 1). Even though the capacity of neutralizing the DPPH free radical of fatty acids is not very relatively high as other antioxidants, the CO and HO of *T. catappa seed* in this study were shown to be 2.5-7 folds higher in this activity compared to the n-hexane extracted oil (28). Moreover, these extraction methods have been proved to be better than the seed supercritical CO_2 extraction, which resulted in no exhibition of DPPH scavenging activity (27).

Conclusion

In recent years, *T. catappa seed* has got increased attention for its nutritional value as well as its potential to be used as alternative biodiesel or dietary lipid (3, 6, 7, 26, 28). This study has provided a detailed fatty acid profile of seed oils extracted at a cold and hot temperature from *T. catappa collected* in Vietnam. The data showed that a high level of unsaturated fatty acids was found in the oils as well as eicosadienoic acid (C20:2, cis-11,14), a valuable fatty acid that has not been detected in previous studies. In addition, seed oils extracted by hot-pressed and cold-press methods had diverse antibacterial activity against both Gram-positive and negative bacteria and displayed considerable DPPH radical scavenging activity compared to those from n-hexane and supercritical CO_2 extraction methods. In conclusion, hot-pressed and especially cold-pressed, could be affordable and readily available methods to obtain good quality *T. catappa seed* oil and the oil could potentially be used for food applications, in pharmaceutical industries, as well as in other related fields.

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Authors contributions

Le Hong Nguy designed this study. All authors searched and handled the data. Le Hong Nguy and Thi Anh Dao Dong drafted the manuscript and resolved all the queries of editors and reviewers.

Compliance with ethical standards

Conflict of interest: No conflict of interest was declared by the authors.

Ethical issues: None.

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