Chemical composition and antioxidant activity of the fruit essential oil of *Xanthium strumarium* L. cultivated in Hai Duong province, Vietnam

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Abstract:

***Xanthium strumarium* L., a species in the *Asteraceae* family, is well known for its use in traditional medicine. This study aimed to identify the chemical composition and evaluate the antioxidant activity of the essential oil (EO) extracted from the fruits of *X. strumarium* collected in Hai Duong province, Vietnam. The EO was obtained via steam distillation and analysed using gas chromatography-mass spectrometry (GC-MS). The results revealed 15 major compounds accounting for 93.88% of the total oil content. The dominant constituents included *m*-tolualdehyde (12.11%), 1,2-benzenedimethanol (11.87%), *p*-tolualdehyde (9.94%), caryophyllene oxide (9.28%), and endo-borneol (8.91%). The major chemical groups were oxygenated sesquiterpenes (26.79%) and aromatic compounds (39.72%). The antioxidant activity of the EO was assessed using three methods: ferricyanide reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-Azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays. The EO demonstrated strong radical scavenging activity, comparable to ascorbic acid, with IC_{50} values of 22.8 µg/ml (DPPH) and 22.1 µg/ml (ABTS). These findings indicate that the fruit EO of *X. strumarium* possesses a rich chemical profile and significant antioxidant potential, suggesting promising applications in pharmaceuticals and functional foods.**

Keywords: **caryophyllene oxide, common cocklebur, endo-borneol, essential oil, radical scavenging.**

Classification numbers: **2.2, 3.1, 3.3, 3.6**

1. Introduction

Xanthium strumarium L., commonly known as cocklebur, is an annual herbaceous plant belonging to the *Asteraceae* family. Native to Central and South America, it has since become naturalised and widely distributed across various countries, including Brazil, China, Malaysia, and the warmer regions of India and Japan [1-3]. *X. strumarium* grows wild in many places in Vietnam (wasteland, field edges, roadside). During harvest, people collect the entire plant except for the roots, which are dried or sun-dried, or they simply harvest the ripe fruits and then dry them or use a drying process. The cocklebur fruits are harvested before turning yellow and then dried or sun-dried [4-6].

Traditionally, *X. strumarium* has been used in various folk medicine systems for treating ailments such as toothache, sore throat, rhinitis, goitre, night sweats, fevers, and rheumatism [5-6]. Its fruits, often dried and powdered, are incorporated into topical formulations to treat skin conditions including abscesses, eczema, leprosy, scabies, and insect bites [7-9]. The leaves have diuretic properties and are used in the treatment of syphilis and scrofula, while root extracts are applied to ulcers and for malaria treatment [8, 10].

The chemical composition of *X. strumarium* EO is known to vary depending on geographical origin, plant part used, and extraction method. Numerous studies have identified major components such as limonene, borneol, *α*-ionone, cis-*β*-guaiene, *β*-caryophyllene, carveol, and *p*-cymene [8, 11-13]. Reported concentrations vary significantly, with *β*-caryophyllene (17.53%), *α*-cadinol (6.66%), spathulenol (6.09%) [10], cis-*β*-guaiene (34.2%), limonene (20.3%), borneol (11.6%), and L-bornyl acetate (4.5%) [12] among the notable constituents. A. Shkondrov, et al. (2021) [14] additionally identified isovalencenol (14.9%) and himachalol (15.0%) as prominent compounds.

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Although a few studies have characterised the EO composition of *X. strumarium*, there remains a lack of data on the EO derived from *X. strumarium* fruits in Vietnam. Therefore, this study aims to: (1) Extract EO from the fruits of *X. strumarium* collected in Hai Duong province, Vietnam, using hydrodistillation; (2) Analyse its chemical constituents using GC-MS; (3) Evaluate its antioxidant potential using the ferricyanide reducing power assay, as well as DPPH and ABTS radical scavenging assays.

2. Materials and methods

2.1. Materials and chemicals

The fruits of *X. strumarium* were collected in November 2023 in Hai Duong province, Vietnam (21°11′16″N 106°25′38″E). The plant material was identified by Assoc. Prof. Dr Pham Thanh Huyen of the National Institute of Medicinal Materials. A voucher specimen, DV-140223, was deposited in the Department of Medicinal Resources at the National Institute of Medicinal Materials, Hanoi, Vietnam. DPPH, potassium persulfate, Tween-80, and potassium ferricyanide ($K_3[Fe(CN)_6]$) were obtained from Sigma-Aldrich (St. Louis, MO, USA), while ABTS and ascorbic acid were purchased from Merck (Darmstadt, Germany). All other chemicals and solvents used in this study were of analytical grade.

2.2. Hydrodistillation of essential oil

Fresh fruits of *X. strumarium* (2 kg) were washed with tap water for surface cleaning, air-dried, and ground into a fine powder. The powdered material was then subjected to hydrodistillation using a Clevenger-type apparatus for 3 hours, repeated in triplicate under identical conditions. The EOs obtained from each batch were combined, dried over anhydrous sodium sulphate, and stored in sealed amber vials at 4°C in the dark until further analysis. The yield of EO was calculated using the following equation (1).

$$\text{Yield of EO (\%)} = \text{Volume of EO/Weight of fruit material} \times 100 \quad (1)$$

2.3. Gas chromatography-mass spectrometry analysis

For GC-MS analysis, 10 µl of *X. strumarium* fruit EO was diluted in 1 ml of *n*-hexane and homogenised using a vortex mixer. The resulting solution was analysed using a Shimadzu GCMS-QP2020 system equipped with a Shimadzu SH Rxi-5Sil MS capillary column (30 m×0.32 mm, 0.25 µm film thickness; stationary phase: 100% dimethyl polysiloxane). The sample was injected at 180°C with a split ratio of 20:1. Helium served as the carrier gas at a flow rate of 1.61 ml/min, and the column pressure was maintained at 21.6 kPa (3.13 psi). The oven temperature programme was as follows: initial temperature at 60°C for 3 minutes, then increased to 120°C at 4°C/min, followed by a rise to 140°C at 5°C/min (held for 4 minutes), and finally ramped to 180°C at 10°C/min with a final hold of 2 minutes. The total GC run time was 35 minutes, with a solvent cut time of 2 minutes.

Mass spectrometric detection was performed using an electron ionisation (EI) source at 200°C and an interface temperature of 250°C. Data were acquired in full scan mode over a mass range of 50-900 m/z. Retention indices were calculated using a homologous series of C_7-C_{30} *n*-alkanes (Aldrich Chemical Co., USA) under identical conditions. Compounds were identified by comparing their mass spectra and retention indices with those in the NIST 08 and Wiley 9th edition libraries, and by referencing published data [15, 16]. The relative percentages of individual components were calculated based on peak areas from the total ion chromatogram (TIC), without the use of correction factors.

2.4. Reducing power assay

The antioxidant activity of *X. strumarium* fruit EO was evaluated based on its reducing power, following the method described by M. Oyaizu (1986) [17] and modified by T.H. Luong, et al. (2022) [18]. Various concentrations of the EO (6.25, 12.5, 25, 50, 100, 150, and 200 µg/ml) were prepared and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide ($K_3[Fe(CN)_6]$). The mixtures were incubated at 50°C for 20 minutes. After incubation, 2.5 ml of 10% (w/v) trichloroacetic acid was added, followed by centrifugation at 3000×g for 20 minutes. Subsequently, 2.5 ml of the resulting supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride solution. The absorbance of the final mixture was measured at 700 nm using a BioTek MicroFill microplate dispenser. An increase in absorbance indicated higher reducing power, reflecting stronger antioxidant activity. Ascorbic acid, a well-known antioxidant, was used as a reference standard for comparison.

2.5. Determination of antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of *X. strumarium* fruit EO was evaluated

using a spectrophotometric method based on G. Miliauskas, et al. (2004) [19], with slight modifications as outlined by D.M.C Nguyen, et al. (2023) [20]. The EO was first dissolved in 0.004% methanol containing 0.5% Tween-80 to enhance solubility. A stock solution was prepared at 1 mg/ml, and working solutions were serially diluted to obtain final concentrations of 6.25, 12.5, 25, 50, 100, 150, and 200 µg/ml.

For each assay, 0.1 ml of EO solution at each concentration was mixed with 1.0 ml of 0.2 mM DPPH solution prepared in 0.004% methanol. The reaction mixture was incubated in the dark at 25±1°C for 15 minutes. Absorbance was measured at 517 nm using a BioTek MicroFill plate reader (Agilent, USA). A control sample containing all reagents except the EO served as the blank. The DPPH radical scavenging activity (%) was calculated using the following equation (2).

$$\text{DPPH radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (2)$$

where $Abs_{control}$ is the absorbance of the control solution and Abs_{sample} is the absorbance of the sample solution. Ascorbic acid was used as a positive control. All assays were conducted in duplicate with three replicates per concentration.

The ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity was evaluated using the method of D.M.C. Nguyen, et al. (2023) [20], with minor modifications. The $ABTS^{+}$• radical cation was generated by reacting 7.4 mM ABTS with 2.6 mM potassium persulfate and incubating the mixture in the dark at room temperature for 12 hours. Before use, the solution was diluted with methanol to obtain an absorbance of 0.70±0.02 at 734 nm. Absorbance was then measured at 517 nm using a BioTek MicroFill plate reader (Agilent, USA).

The EO was prepared as described for the DPPH assay and tested at concentrations of 6.25, 12.5, 25, 50, 100, 150, and 200 µg/ml. In the assay, 0.2 ml of each diluted sample was added to 1.8 ml of $ABTS^{+}$• solution. After incubation in the dark at 25±1°C for 2 hours, the absorbance was measured at 734 nm. The scavenging activity (%) was calculated using equation (3).

$$\text{ABTS radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (3)$$

where $Abs_{control}$ is the absorbance of the $ABTS^{+}$• solution without the sample, and Abs_{sample} is the absorbance of the solution containing the EO. Ascorbic acid was used as a reference standard. All experiments were repeated twice with triplicate measurements per treatment.

The half-maximal inhibitory concentration (IC_{50}) values for both DPPH and ABTS radical scavenging activities were calculated to evaluate the antioxidant potential of the EO.

2.6. Statistical analysis

Data were compared using Tukey's honestly significant difference (HSD) test, with $p \leq 0.05$ indicating a significant difference among mean values. All data were analysed using Statistical Analysis System 9.1 [21] and are presented as mean ±SD.

3. Results and discussion

3.1. Chemical composition of the fruit essential oil of X. strumarium

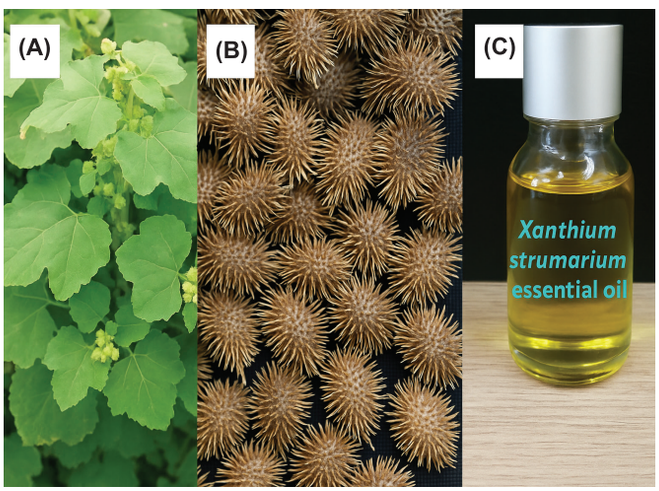

Fig. 1. Morphological characteristics of (A) the plant of *X. strumarium*, (B) the dried fruit of *X. strumarium* (Hai Duong province, Vietnam), and (C) its essential oil extracted by hydrodistillation.

The fruits are oval in shape, measuring approximately 1-2 cm in length and 0.5-1 cm in width. They possess a hard outer shell, which is green when immature (Fig. 1A) and turns brown upon maturation (Fig. 1B). The EO extracted from the fruit is bright yellow in colour and emits a mild, characteristic aroma (Fig. 1C). The average yield was 0.11±0.02% (v/w).

Table 1. Chemical composition of fruit essential oil of *X. strumarium* by gas chromatography-mass spectrometry analysis.

Peak	Compounds	RT (min)	Retention indices		Content (%)
			RI_{Db}	RI_{Cal}	
1	Isocumene	5.37	1508±5	1512	3.78
2	m-Ethylmethylbenzene	5.56	1517±2	1519	3.17
3	D-Limonene	7.89	1528±7	1531	6.33
4	Benzeneacetaldehyde	8.38	1537±4	1540	1.85
5	1,2-Benzenedimethanol	9.38	1548±3	1549	11.87
6	m-Tolualdehyde	9.55	1552±2	1555	12.11
7	p-Tolualdehyde	10.13	1558±3	1559	9.94
8	Endo-Borneol	14.55	1679±8	1571	8.91
9	Eugenol	25.13	1588±7	1593	3.76
10	Humulene	31.11	1609±7	1612	2.99
11	β-copaene	32.66	1622±8	1625	2.28
12	Germacrene B	37.05	1639±5	1641	3.10
13	Caryophyllene oxide	38.33	1650±4	1652	9.28
14	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	39.90	1661±5	1663	8.64
15	Shyobunol	44.51	1677±6	1679	8.87
Monoterpene hydrocarbons (peak Nos. 3)					6.33
Oxygenated monoterpenes (peak Nos. 8-9)					12.67
Sesquiterpene hydrocarbons (peak Nos. 10-12)					8.37
Oxygenated sesquiterpenes (peak Nos. 13-15)					26.79
Diterpenes					0
Other					39.72
Total identified					93.88

RT = Retention time (min)

RI_{Cal}: Retention indices calculated with standard non-polar GC column in library spectra NIST 17 (Software NIST MS search v2.3)

RI_{Db} (Column Rxi-1 MS): Retention indices from the database.

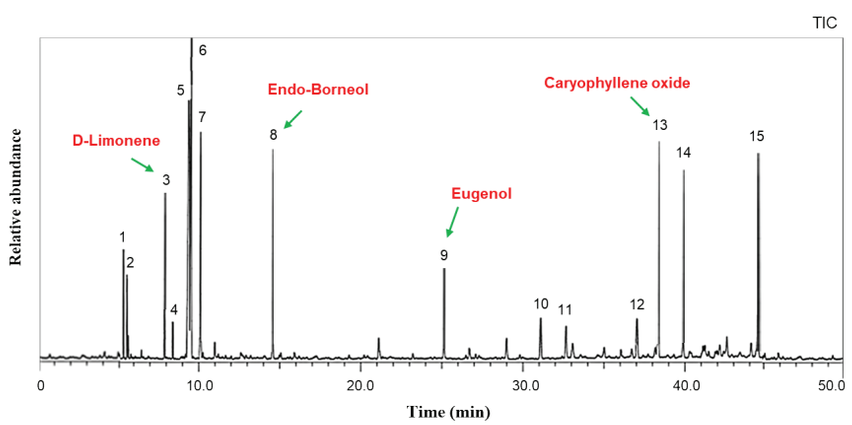

Fig. 2. Gas chromatography-mass spectrometry profile of the compounds in the fruit essential oil of *X. strumarium*. The chemical structures of the main compounds are shown in the figure.

Gas chromatography-mass spectrometry analysis of the EO extracted from the fruit of *X. strumarium* revealed 15 identified compounds, accounting for 93.88% of the total oil content (Table 1, Fig. 2). The chemical composition included various classes of terpenoids and aromatic compounds. The major constituents were *m*-tolualdehyde (12.11%), 1,2-benzenedimethanol (11.87%), *p*-tolualdehyde (9.94%), caryophyllene oxide (9.28%), endo-borneol (8.91%), shyobunol (8.87%), and D-limonene (6.33%). Minor yet notable components included eugenol (3.76%), isocumene (3.78%), *m*-ethylmethylbenzene (3.17%), and various sesquiterpenes such as humulene, *β*-copaene, and germacrene B.

A study by A. Shkondrov, et al. (2021) [14] in Bulgaria reported that the EO was predominantly composed of terpenes (52.36%), with sesquiterpenes isovalencenol (14.89%) and himachalol (14.98%) as principal constituents. In contrast, the current study shows a different quantitative profile, in which oxygenated sesquiterpenes were the most abundant class (26.79%), followed by aromatic aldehydes and alcohols (39.72%), oxygenated monoterpenes (12.67%), monoterpene hydrocarbons (6.33%), and sesquiterpene hydrocarbons (8.37%). This composition indicates a high proportion of oxygenated compounds, often linked to enhanced biological activity.

In Iran, S. Ghahari, et al. (2017) [22] identified thirty-six EO compounds accounting for 97.89% of total oil content, with methyl linoleate (40.64%), methyl oleate (13.12%), and methyl palmitate (12.43%) as dominant constituents. In Bulgaria, sixteen previously unreported compounds were identified, including sesquiterpenes such as humulene-1,2-epoxide (5.51%), modephene (3.56%), *α*-isocomene (1.73%), and *α*-bisabolol oxide (1.23%); a sterol, 3-hydroxyspirost-8-en-11-one (0.80%); four alkanes; an ester, (8Z)-7-methyl-8-tetradecenyl acetate (0.64%); and an acetal, 1-methylene-2-(4,4-diethoxybutyl)cyclopropane (3.64%) [14].

In Egypt, the EO from *X. strumarium* leaves was found to contain forty-three compounds [13], with sesquiterpenoids being dominant (72.40%). These included oxygenated

Table 2. Radical-scavenging activity and reducing power of the essential oil obtained from *X. strumarium*.

Concentration (µg/ml)	DPPH radical-scavenging activity (%)		ABTS radical-scavenging activity (%)		Reducing power (△OD)	
	X. strumarium	*Ascorbic acid*	*X. strumarium*	*Ascorbic acid*	*X. strumarium*	*Ascorbic acid*
6.25	18.9±0.6^{g}	22.1±1.3^{f}	21.8±2.3^{e}	24.6±0.6^{e}	0.04±0.01^{e}	0.05±0.01^{e}
12.5	39.3±0.7^{f}	42.3±1.1^{e}	42.2±1.7^{d}	45.5±1.8^{d}	0.09±0.00^{d}	0.09±0.00^{d}
25	66.5±0.5^{e}	70.8±0.4^{d}	65.6±1.2^{c}	69.1±0.8^{c}	0.20±0.01^{c}	0.20±0.01^{c}
50	75.8±0.2^{d}	77.8±0.4^{c}	81.1±1.1^{b}	81.4±1.2^{b}	0.43±0.01^{b}	0.45±0.01^{b}
100	89.9±0.4^{c}	89.2±0.4^{b}	94.5±0.3^{a}	94.1±0.4^{a}	0.90±0.01^{a}	0.93±0.01^{a}
150	93.0±0.4^{b}	94.2±0.3^{a}	94.8±0.3^{a}	95.1±0.4^{a}	1.07±0.01^{a}	1.10±0.01^{a}
200	94.7±0.3^{a}	95.6±0.3^{a}	95.4±0.3^{a}	95.9±0.2^{a}	1.12±0.01^{a}	1.13±0.01^{a}
IC_{50} (µg/ml)	22.8±1.6	21.7±1.1	22.1±1.2	20.7±1.4	-	-

△OD: Change in absorbance at 700 nm.

sesquiterpenes (61.78%), non-oxygenated sesquiterpenes (10.62%), and monoterpenes (25.19%). Diterpenoids and oxygenated hydrocarbons were minor components. The main constituents were 1,5-dimethyltetralin (14.27%), eudesmol (10.60%), *l*-borneol (6.59%), ledene alcohol (6.46%), (-)-caryophyllene oxide (5.36%), isolongifolene-7,8-dehydro-8a-hydroxy (5.06%), L-bornyl acetate (3.77%), and aristolene epoxide (3.58%).

These findings suggest that the chemical composition of *X. strumarium* collected in Hai Duong province, Vietnam, differs markedly from those reported in Bulgaria [14], Egypt [13], and Iran [22], likely due to geographic, climatic, and ecological variations affecting the plant's secondary metabolism [7, 23].

The chromatographic profile (Fig. 2) showed intense peaks corresponding to the most abundant constituents, with peaks 5-7 (aromatic alcohols and aldehydes), peak 13 (caryophyllene oxide), and peak 15 (shyobunol) standing out prominently. The chemical profile of *X. strumarium* fruit EO reveals a diverse blend of terpenoids and aromatic compounds, many of which are known for their bioactivity. The dominant presence of aromatic aldehydes such as *m*- and *p*-tolualdehyde, along with 1,2-benzenedimethanol, is noteworthy, as these compounds are less frequently reported as major constituents in EOs and may contribute to the distinct fragrance and biological properties of this oil.

The relatively high content of oxygenated sesquiterpenes (26.79%) - particularly caryophyllene oxide, shyobunol, and a bicyclic ether compound (peak 14) - suggests potential anti-inflammatory, antimicrobial, and antioxidant activity, as reported in other EO studies [24, 25]. Additionally, D-limonene (6.33%), identified at peak 3, is a well-known monoterpene with proven anti-cancer, insecticidal, and antioxidant effects [12]. Its presence alongside endo-borneol (8.91%) and eugenol (3.76%) enhances the oil's potential therapeutic value, particularly in applications involving microbial inhibition and inflammation control. The EO from *X. strumarium* fruits has a distinct chemical composition compared to oils from other plant parts, likely due to tissue-specific biosynthesis and developmental factors [26, 27]. Overall, the high percentage of oxygenated compounds (39.46%) supports the hypothesis that the fruit EO of *X. strumarium* may possess strong biological activities, justifying further investigation into its antimicrobial, anti-inflammatory, and antioxidant potential.

3.2. Reducing power of the fruit essential oil of X. strumarium

The results are expressed as the mean ±SD from triplicate measurements. Different letters in the column indicate a significant difference at each observation time based on a Tukey's HSD test with significance $p \leq 0.05$.

The reducing power assay evaluates a substance's ability to donate electrons to reduce oxidised species, providing additional insight into its antioxidant properties. In this assay, *X. strumarium* EO demonstrated a dose-dependent increase in reducing power, starting at 0.04±0.01 at 6.25 µg/ml and reaching 1.12±0.01 at 200 µg/ml. Ascorbic acid showed a similar trend, with reducing power ranging from 0.05±0.01 to 1.13±0.01 at the same concentrations (Table 2). These results indicate that *X. strumarium* EO not only scavenges free radicals but also effectively donates electrons, thereby enhancing its antioxidant potential. The strong reducing

activity observed supports the EO's potential role in mitigating oxidative stress. Notably, this finding aligns with previous reports demonstrating the significant antioxidant activity of *X. strumarium* EO [13, 22], and the EO's reducing power in this study was comparable to or greater than that of several other plant-derived EOs documented in the literature.

3.3. Antioxidant activity of the fruit essential oil of X. strumarium

The DPPH assay measures the ability of a substance to donate hydrogen atoms to neutralise free radicals. As shown in Table 2, the DPPH radical scavenging activity of *X. strumarium* EO increased significantly with concentration. At 6.25 µg/ml, the scavenging activity was 18.9±0.6%, rising to 94.7±0.3% at 200 µg/ml. Ascorbic acid exhibited a similar trend, with a scavenging activity of 95.6±0.3% at the highest concentration. These findings suggest that *X. strumarium* EO has strong radical-scavenging properties, comparable to ascorbic acid, particularly at higher concentrations. The increase in activity with concentration further highlights the dose-dependent nature of its antioxidant effect.

The ABTS assay, another widely used method to assess antioxidant capacity, showed a similar trend in radical scavenging for *X. strumarium* EO. At 6.25 µg/ml, *X. strumarium* EO exhibited 21.8±2.3% scavenging activity, which increased to 95.4±0.3% at 200 µg/ml (Table 2). The activity of *X. strumarium* EO was nearly identical to ascorbic acid, which exhibited 95.9±0.2% at the same concentration (Table 2). This parallel behaviour in both DPPH and ABTS assays further confirms that *X. strumarium* EO possesses potent antioxidant activity capable of effectively neutralising free radicals.

The IC_{50} value represents the concentration required to inhibit 50% of the radical species in the assays. The IC_{50} values for *X. strumarium* EO were found to be 22.8 µg/ml for DPPH (Fig. 3A; y=1.7554x+9.9698; R^2=0.9267) and 22.1 µg/ml for ABTS (Fig. 3B; y=1.7441x+11.429; R^2=0.9250). These values were comparable to those of ascorbic acid, which exhibited IC_{50} values of 21.7 µg/ml for DPPH (Fig. 3A; y=1.7367x+12.247; R^2=0.9011) and 20.7 µg/ml for ABTS (Fig. 3B; y=1.7438x+13.594; R^2=0.9037). These results suggest that *X. strumarium* EO is an effective antioxidant, with IC_{50} values similar to those of a known antioxidant such as ascorbic acid. The lower the IC_{50} value, the more potent the antioxidant; these values indicate that

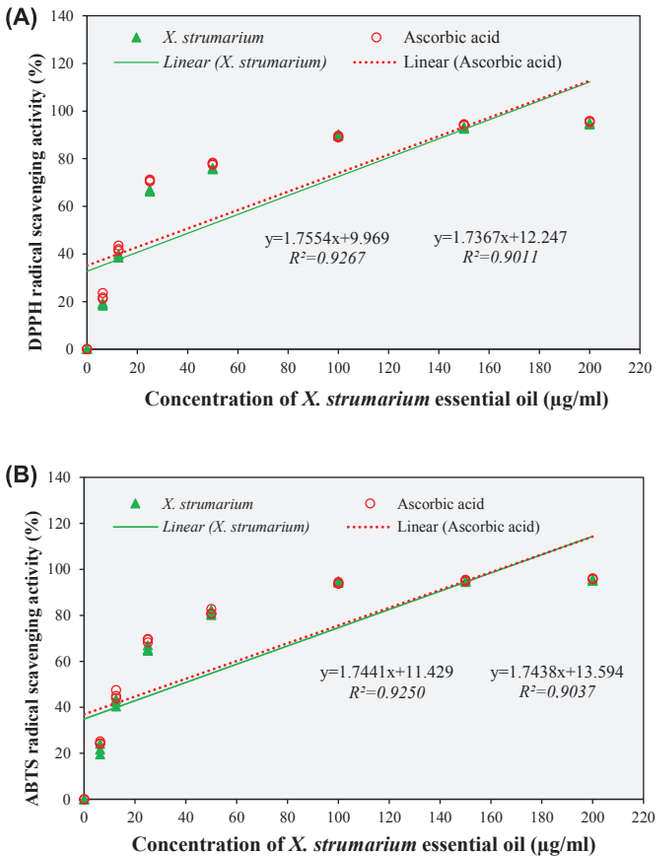

Fig. 3. Radical scavenging activity (%) of *X. strumarium* essential oil at various concentrations with corresponding linear regression equations for (A) DPPH and (B) ABTS assays. Ascorbic acid (AA) was used as the reference standard. IC_{50} values for DPPH, ABTS, and AA were calculated based on the regression curves.

X. strumarium EO can effectively neutralise oxidative stress at relatively low concentrations. S. Ghahari, et al. (2017) [22] reported that the EO extracted from the fruits of *X. strumarium* exhibited significant DPPH radical scavenging activity, with an IC_{50} value of 138.87 µg/ml. In contrast, A.A.E. Gawad, et al. (2019) [13] found that the EO from *X. strumarium* leaves demonstrated weaker DPPH radical scavenging activity, with an IC_{50} of 321.93 µg/ml, compared to ascorbic acid, which had an IC_{50} of 35.07 µg/ml.

Phytochemical analyses have revealed that *X. strumarium* EOs are predominantly composed of terpenes, including significant amounts of sesquiterpenes such as isovalencenol and himachalol [14]. According to recent reports, EOs from medicinal plants play several biological roles, including antioxidative or free radical scavenging activities. The results of this study underscore the significant antioxidant potential of *X. strumarium* EO. The EO

demonstrated strong radical-scavenging activity, with both DPPH and ABTS assays showing dose-dependent increases in antioxidant capacity. This is consistent with previous studies on other *Xanthium* species, which have reported high levels of flavonoids and terpenes known for their antioxidant properties [13, 22, 27]. Given these findings, future research should focus on isolating and quantifying the specific bioactive constituents within *X. strumarium* EO to better understand their individual contributions to its antioxidant capacity. Such studies could pave the way for the development of natural antioxidant agents derived from this plant.

4. Conclusions

The present study successfully extracted and analysed the EO from the fruits of *X. strumarium* collected in Hai Duong province, Vietnam. GC-MS analysis identified fifteen chemical constituents, accounting for 93.88% of the total oil composition. The oil was rich in aromatic compounds (39.72%) and oxygenated sesquiterpenes (26.79%), with major constituents including *m*-tolualdehyde, 1,2-benzenedimethanol, *p*-tolualdehyde, caryophyllene oxide, endo-borneol, shyobunol, and D-limonene. This distinct chemical profile, especially the high levels of oxygenated compounds, suggests notable biological activity. The EO exhibited strong and dose-dependent antioxidant activity, with performance comparable to that of ascorbic acid at higher concentrations. These findings highlight the potential of *X. strumarium* fruit EO as a natural source of antioxidant agents and justify further investigation for pharmaceutical or cosmetic applications. Future studies should explore the antimicrobial, anti-inflammatory, and cytotoxic potential of individual constituents, as well as conduct *in vivo* experiments to validate the bioactivity and safety of this EO.

CRediT author statement

Dang-Minh-Chanh Nguyen: Conceptualisation, Methodology, Data analysis, Software, Data curation, Investigation, Original draft preparation, Writing; Thi-Hoan Luong: Conceptualisation, Methodology, Data analysis, Writing - Reviewing and Editing.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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