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Research article

In vitro lipolysis and physicochemical characterization of unconventional star anise oil towards the development of new lipid-based drug delivery systems

Jannet Kamoun [a,b], Fatma Krichen [c], Imed Koubaa [d], Nacim Zouari [e], Ali Bougatef [c], Abdelkarim Abousalham [b], Ahmed Aloulou [a,*]

[a] University of Sfax, National Engineering School of Sfax, Laboratory of Biochemistry and Enzymatic Engineering of Lipases, Sfax 3038, Tunisia
[b] Univ Lyon, Université Lyon 1, Institut de Chimie et de Biochimie Moléculaires et Supramoléculaires (ICBMS), UMR 5246 CNRS, Génie Enzymatique, Membranes Biomimétiques et Assemblages Supramoléculaires (GEMBAS), Bât Raulin, 43 Bd du 11 Novembre 1918, F-69622 Villeurbanne Cedex, France
[c] University of Sfax, National Engineering School of Sfax, Laboratory of Plant Improvement and Valorization of Agro-resources, Sfax 3038, Tunisia
[d] University of Sfax, Faculty of Science of Sfax, Laboratory of Organic Chemistry, Sfax 3038, Tunisia
[e] University of Gabes, Higher Institute of Applied Biology of Medenine, Medenine 4119, Tunisia

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ABSTRACT

Lipid-based drug delivery systems are widely used for enhancing the bioavailability of poorly water-soluble drugs. However, following oral intake, lipid excipients often undergo gastrointestinal lipolysis, which drastically affects drugs solubility and bioavailability. That's why developing new lipid excipients which are resistant to digestion would be of great interest. We studied here the potential role of the unconventional Chinese star anise whole seedpod oil (CSAO) as an alternative multifunctional lipid excipient. Pancreatic lipase-mediated digestion of the extracted crude oil emulsion was assessed *in vitro*. Pancreatic lipase, being a strict *sn*-1,3-regioselective lipase, showed a high (16-fold) olive oil to CSAO activity ratio, which could be attributed to fatty acids composition and triglycerides intramolecular structure. For the sake of comparison, the non-regioselective lipase Novozyme® 435 exhibited higher activity than pancreatic lipase on CSAO emulsion, perhaps due to its ability to release fatty acids from the internal *sn*-2 position of TAGs. Apart counteracting lipolysis, CSAO oil also showed additional biopharmaceutical benefits including moderate antioxidant and antihypertensive activities. Altogether, these findings highlight for the first time the potential use of star anise unconventional whole seedpod oil as a multifunctional lipid excipient for the development of new lipid formulations.

1. Introduction

Drugs formulation is carried out with the main objective of enhancing their bioavailability. Lipid-based drug delivery systems showed great potential in overcoming problems of low bioavailability of poorly soluble drugs. Encapsulating lipophilic drugs in lipid excipients might lead to increased solubilization and prevent precipitation during the gastrointestinal passage, thus resulting in enhanced absorption and bioavailability of poorly soluble drugs. Recent advances in these formulation technologies have led to the successful commercialization of lipid-based drug delivery systems [1]. There is a growing interest in understanding lipid formulations digestibility in the gastrointestinal tract before designing lipid-based drug delivery systems. Many studies are currently working on the development of *in vitro* models for assessing drug bioavailability [1]. Lipid formulations currently used for the oral delivery of poorly water-soluble drugs consist of esters, such as acylglycerols, phospholipids, polyethyleneglycol esters and polysorbate, that could be hydrolysed by gastrointestinal lipolytic enzymes, which might affect poorly water-soluble drugs bioavailability [2].

Developing a mechanistic understanding of the impact of food structure and composition on human health has increasingly involved simulating digestion in the upper gastrointestinal tract. Simulated gastrointestinal digestion is widely employed in many fields of food and nutritional sciences, as conducting human trials are often costly, resource intensive, and ethically disputable. Therefore, simple *in vitro* digestion models mimicking the gastrointestinal tract have been proposed as alternatives to *in vivo* experiments. Despite the simplicity of *in vitro*

* Corresponding author.
E-mail address: ahmed.aloulou@enis.tn (A. Aloulou).

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digestion models they are often very useful in predicting outcomes of the digestion *in vivo* [3].

Nowadays research is focused on the identification of unconventional oils, that have unique health-promoting properties or functional characteristics, to meet the rising global need for edible oils. Unconventional oils are a promising alternative that would extend the use of oils in pharmaceutical, food, cosmetic and energy industries [4, 5]. However, unconventional oils should overcome certain challenges to ensure sustainable intensive production.

Chinese star anise (CSA), the seedpod of an evergreen tree (*Illicium verum*) grown in East Asia, is an edible fruit that has been commonly regarded as being safe and nontoxic. It is widely used as a spice and in traditional medicine [6, 7]. Most previous studies were focused on the fruit peel essential oil, but there is not enough information on the whole seedpod unconventional oil of Chinese star anise (CSAO). The main objective of this work is to assess the *in vitro* gastrointestinal lipolysis of unconventional CSAO oil for the development of new lipid-based drug delivery systems. Moreover, we studied the ability of CSAO to exhibit additional pharmacological properties, e.g., antioxidant and antihypertensive activities, that might strengthen its potential role as a multifunctional lipid excipient.

2. Results and discussion

2.1. Extraction yield of the unconventional star anise seedpod oil

Considering the disadvantages of the hot extraction process affecting the oil quality, a cold extraction method was used in this study to extract Chinese star anise whole seedpod oil (CSAO). Crude oil yield was 7.5 % (on dry matter basis) which is in close agreement with previous results (6.9 %) under similar conditions [8].

2.2. Physicochemical properties of the unconventional star anise seedpod oil

Lipid composition provides a detailed fingerprint of each oil and allows to evaluate its nutritional quality [9].

The global GC-MS analysis of CSAO oil revealed the presence of trans-anethole (a phenylpropanoid), accounting for 14.84 % of the total lipid content, as well as saturated (28.22 %) and unsaturated (56.94 %) fatty acids (Table 1). It has been shown that a high relative level of trans-anethole provides an indication of *I. verum* authenticity [10]. Trans-anethole is known as a popular flavoring substance of commercial value [11].

Table 1. Unconventional CSAO oil analysis by GC-MS technique. Lipid compounds mean percentages were obtained by FID peak-area normalization.

Compounds	Amount (%)
Trans-anethole	14.84
Tetradecanoic acid methyl ester (C14:0)	0.87
Hexadecanoid acid methyl ester (C16:0)	22.95
9-Hexadecanoid acid methyl ester (C16:1)	0.94
Octadecanoic acid methyl ester (C18:0)	4.40
9-Octadecanoic acid methyl ester (C18:1)	30.42
9-Octadecanoic acid methyl ester (C19:1)	0.71
9,12-Octadecanoic acid methyl ester (C18:2)	23.32
9,12,15-Octadecanoic acid methyl ester (C18:3)	1.54
Phenylpropanoids	14.84
Total fatty acids	85.16
Total saturated fatty acids	28.22
Total unsaturated fatty acids	56.94
Mono-unsaturated fatty acids	32.08
Poly-unsaturated fatty acids	24.86

CSAO has a considerable level of essential polyunsaturated fatty acids. In the sake of comparison, oleic, linoleic and palmitic acids are the most abundant fatty acids in both CSAO and extra virgin olive oil (EVOO) [12]. CSAO has higher amounts of linoleic (27.38 % *vs.* 16.21 %) and palmitic (26.94 % *vs.* 15.82 %) acids than EVOO. Oleic acid is however less abundant than in EVOO (35.72 % *vs.* 62.37 %).

Neutral and polar lipids from CSAO oil were analyzed qualitatively using TLC methods. Results showed that CSAO total lipids are dominated by neutral lipids (Figure 1) while polar lipids are not detected (data not shown). Neutral lipids TLC profile shows the presence of trans-anethole, TAGs and hydrolysis products traces, especially free fatty acids (FFAs) and diglycerides.

The determination of triacylglycerols (TAGs) was monitored by the equivalent carbon number (ECN) method. As expected, CSAO is characterized by the abundance of long-chain TAGs (Table 2). Similar ECN values are also found in other oleic acid-rich vegetable oils like EVOO [12] and hazelnut oil [13].

Minor components, including FFAs, chlorophylls, carotenoids and phenolic compounds, contribute to vegetable oils quality, authenticity and health benefits [14].

Phenolic compounds are recognized as natural hydrophilic antioxidants which occur naturally in vegetable oils [15]. As shown in Table 3, CSAO total polyphenols content is around 1255 ppm, which is higher than that of olive oil which is known to be unique with its richness in phenolic compounds (500–800 ppm) [15].

Carotenoids and chlorophylls are natural pigments commonly found in vegetable oils giving them their colors [14]. A high-carotenoids diet is

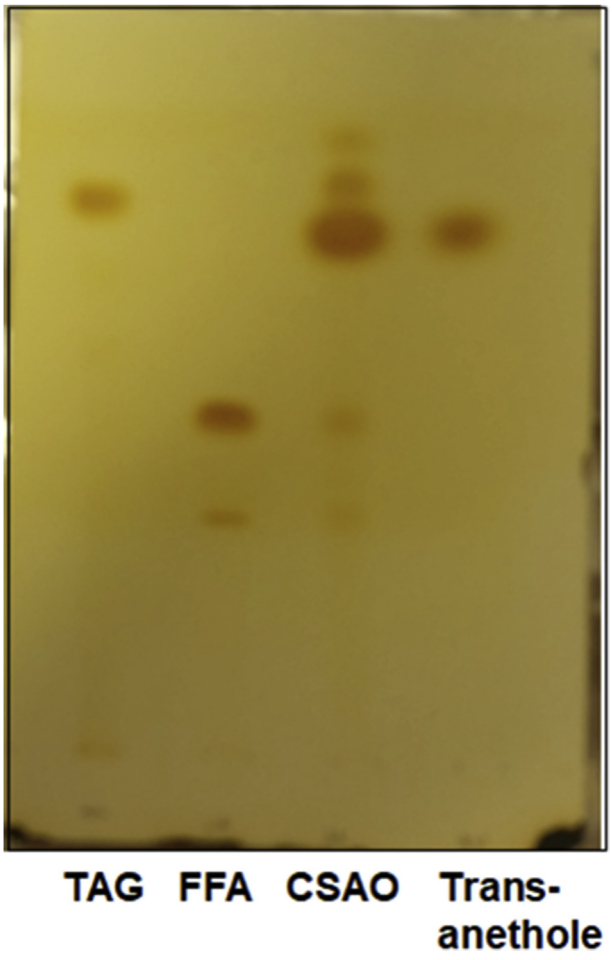

Figure 1. TLC analysis of unconventional Chinese star anise whole seedpod oil (CSAO) neutral lipids as revealed by staining with iodine. TAG, FFA and trans-anethole were included as standards.

Table 2. Triacylglycerols composition of unconventional Chinese star anise whole seedpod oil (CSAO) based on equivalent carbon number (ECN).

TAGs	Amount (%)
ECN40	2.46
ECN42	5.37
ECN44	21.37
ECN46	30.34
ECN48	33.58
ECN50	6.89

Table 3. Polyphenols, tocopherols and pigments concentrations in unconventional Chinese star anise whole seedpod oil (CSAO).

Minor components	Amount (ppm)
Carotene	18.6 ± 0.2
Chlorophylls	16.1 ± 0.1
Polyphenols (GAE)	1255 ± 129
Total tocopherols	21344
α-tocopherol	2313
β-tocopherol	19031

associated with a low incidence of age-related retinal diseases involving pigments antioxidant and provitamin A activities [14]. The CSAO carotenoids content is 18.6 ppm (Table 3). This concentration is much lower than that found in carotenoids-rich crude palm oil (500–700 ppm) [14], but in the same range as EVOO (4–18 ppm) [9]. The CSAO chlorophylls content is 16.1 ppm (Table 3) which is within the range of chlorophylls-rich olive oil (4–48 ppm) [9].

Spectroscopy is an accurate and rapid way to establish the authenticity of vegetable oils [16]. UV-visible measurements in the range of 230–700 nm show that CSAO UV absorption spectrum is characteristic of trans-anethole with a major peak located at 258 nm (Figure 2A), which is in good agreement with previous trans-anethole analysis [17]. IR spectroscopy is a method that performs a chemical analysis of a sample [16]. CSAO and standard trans-anethole show similar IR spectra at the range of 3800–750 cm^{-1} with varying intensities (data not shown). CSAO spectrum exhibits however distinguishable peaks at the range of 1780–1660 cm^{-1} that can be interpreted as the ester carbonyl of TAGs bearing unsaturated fatty acids (Figure 2B).

CSAO oil is liquid at room temperature and has a greenish-yellow color and a mild sweet odor characteristic of anethole. The CSAO refractive index (1.53) is higher than that of edible vegetable oils (1.4) [18] but slightly lower than that of anethole (1.56) [11]. Refractive index, which is a physical property directly related to the purity of oils, could be employed for the detection of adulteration at levels higher than 10 % [19]. Accordingly, the relatively high refractive index of CSAO, compared to edible vegetable oils, would be attributed to its relatively high content of the naturally occurring trans-anethole (14.84 %, Table 1).

Acidity, an indicator of FFAs content, and the iodine value, reflecting the unsaturation degree, are important parameters to evaluate vegetable oils quality. The CSAO acidity (3.54 % of oleic acid) is higher than the standard threshold (Codex Standards for Fats and Oils), indicating that this oil is not suitable for direct consumption and requires refining process for edible purposes. Olive oil, being rich in monounsaturated fatty acids (C18:1), has iodine values in the range of 75–90, while corn and soybean oils, being rich in polyunsaturated fatty acids (C18:2), have iodine values ranging from 120 to 140 [18]. The CSAO iodine value (16.32) was lower compared to major edible vegetable oils, which might be due to the presence of relatively high levels of trans-anethole along with saturated fatty acids (Table 1).

Thermal stability, especially during cooking process, is of great interest to avoid the deterioration of vegetable oils [20]. The CSAO thermal stability was assessed using thermogravimetry which is a simple, fast and reproducible method [21]. This technique basically allows reproducing

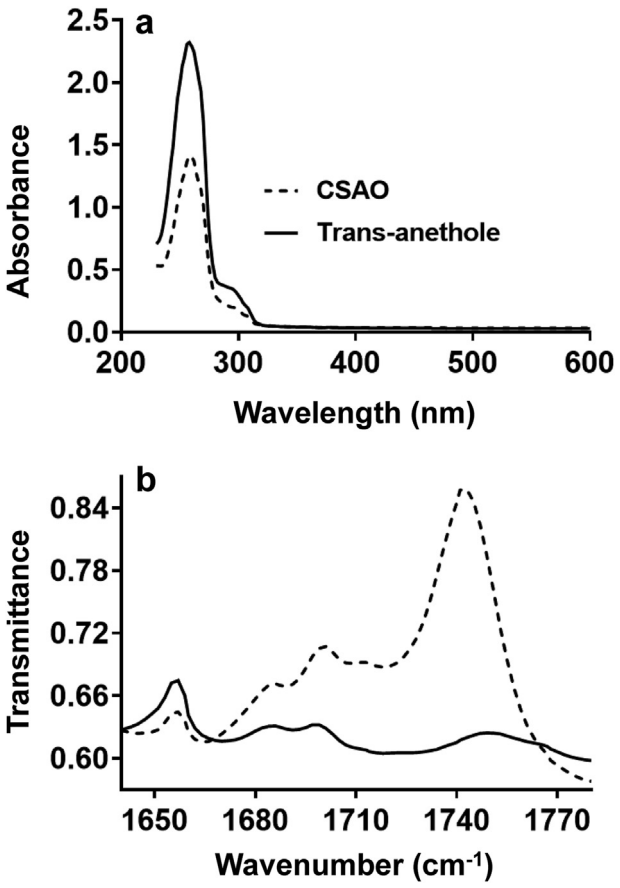

Figure 2. (A) UV-vis absorption spectra of Chinese star anise whole seedpod oil (CSAO) and standard trans-anethole, obtained at 230–700 nm. (B) Expanded FTIR spectral region that exhibits the observed differences between CSAO and standard trans-anethole.

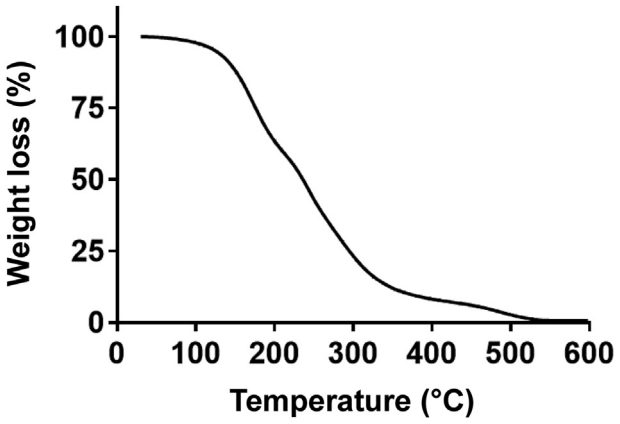

Figure 3. Thermogravimetric curve from Chinese star anise whole seedpod oil (CSAO) under the heating rate of 10 °C/min and 10 mL/min oxygen flow.

the heating process in the oven by continuously monitoring changes in samples weight while the sample is being temperature programmed in an oxygen environment. On the basis of the thermogravimetric analysis (Figure 3), CSAO exhibits lower thermal stability, with a gradual weight loss starting at about 120 °C, than olive (288 °C), sunflower (304 °C) and corn (306 °C) oils [20].

2.3. *In vitro* digestibility of the unconventional star anise seedpod oil

The link between food and human health is increasingly a topic of interest. *In vitro* models have been widely used to overcome the

constrictions associated with *in vivo* methodology [3]. It is generally assumed that pancreatic lipase (PL) is the main enzyme involved in the gastrointestinal lipolysis of oils and fats. PL has been widely used for the *in vitro* simulation of duodenal lipolysis [1]. We studied the *in vitro* pancreatic lipase-mediated lipolysis of CSAO oil emulsion, without any poorly water-soluble drug, in comparison with other vegetable oils emulsions. Based on the initial velocity of TAG hydrolysis, PL specific activity was found to be 335 U/mg on CSAO, i.e. 16-, 8- and 5-fold lower than the maximum activity measured using olive, corn and sunflower oils, respectively, under the same conditions (Figure 4A). PL low specific activity on CSAO would be related to differences in fatty acids composition and TAGs intramolecular structure of investigated oils. In terms of fatty acids composition, the oleic acid, which is predominantly present in EVOO (62.4 %) [12], is known to be rapidly and preferably hydrolyzed by PL [22]. While, the linoleic acid, which was predominantly present in corn and sunflower oils (55.9 % and 61.5 %, respectively) [12], has more steric requirements to be hydrolyzed due to its higher number of unsaturation [22]. In terms of TAG intramolecular structure, OOO is the major TAG species in olive oil and to lesser extent POO and OLO [23]. Accordingly, the predominance of oleic acid in the *sn*-1 and *sn*-3 positions in olive oil TAG could explain the high activity of PL towards EVOO. Similarly, the predominance of linoleic acid in the external positions of the major TAG species of corn and sunflower oils [23] could explain the lower PL activity towards these two vegetable oils when compared to EVOO. Although TAGs species of CSAO are not identified yet, they would certainly differ from the two other investigated vegetable oils models (dominated with oleic and linoleic acids). Accordingly, the resistance of CSAO oil to PL lipolysis would be attributed to the strict *sn*-1, 3-regioselectivity of PL, as well as to the steric hindrance caused by polyunsaturated fatty acids at the TAGs external carboxyl ester bonds [24].

In line with this finding, we checked whether CSAO could be hydrolyzed *in vitro* by the non-regiospecific (or random) *Candida antarctica* B lipase (Novozyme® 435). Based on the initial velocity, Novozyme® 435 exhibited higher activity than pancreatic lipase on CSAO emulsion, the enzyme amount in both cases being equivalent to 10 units on olive oil emulsion. One could plausibly suggest that this might be attributed to Novozyme® 435 ability to release fatty acids from the internal *sn*-2 position of TAGs (Figure 4B). It has been reported that oleic acid occurs mainly at the *sn*-2 position of vegetable TAGs [23]. Therefore, oleic acid, which accounts for 35.72 % of total CSAO fatty acids (Table 1), would rather occupy the internal *sn*-2 position of CSAO TAGs species. Moreover, the olive oil to CSAO activity ratio of Novozyme® 435 was found to be around 1, clearly indicating that pancreatic lipase-mediated lipolysis is not affected by the physical properties of the CSAO emulsion, such as particle size, but rather by fatty acids composition and TAGs intramolecular structure, i.e., the positional distribution of fatty acids on the glycerol backbone, as it has been reported previously [24].

It is assumed that fast lipolysis of triglyceride-based oral lipid formulations by gastric and pancreatic lipases can lead to premature release of poorly water-soluble drugs, and therefore drug precipitation in the gastrointestinal lumen followed by reduced intestinal absorption [25, 26]. Slowing down lipid excipients lipolysis might therefore significantly enhance the bioavailability of lipophilic drugs. However, since lipolysis products, i.e. fatty acids and monoglycerides, are known to trigger certain lipophilic drugs absorption [27], the coformulation with fatty acids and monoglycerides could be a promising strategy for ensuring a better bioavailability of lipophilic drugs.

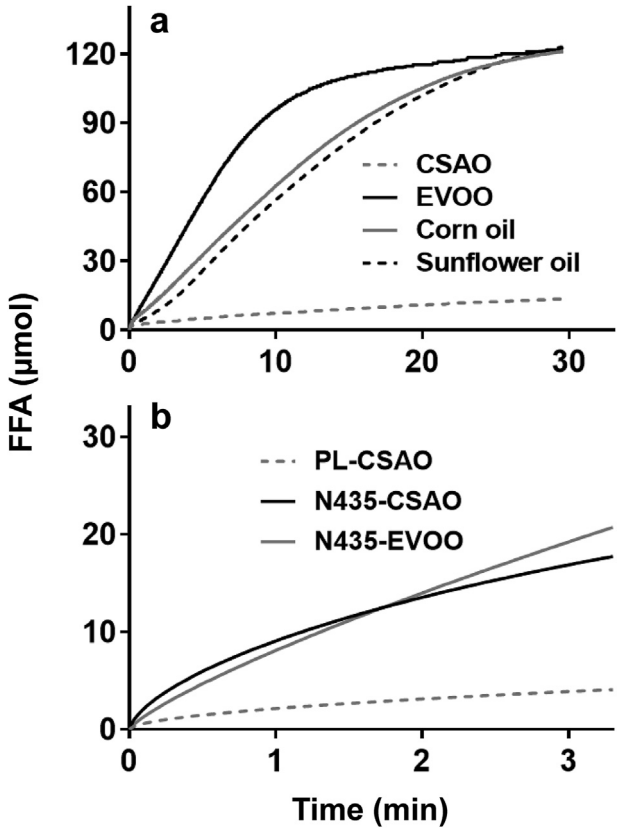

Figure 4. (A) Kinetics of hydrolysis of Chinese star anise whole seedpod, corn, sunflower and olive oils emulsions by pancreatic lipase. (B) Kinetics of hydrolysis of CSAO emulsion by Novozyme® 435 and pancreatic lipase. Novozyme® 435-mediated hydrolysis kinetics of olive oil were recorded as a control experiment. The enzyme amount is equivalent to 10 units on olive oil emulsion in each case.

2.4. Biological activities of the unconventional star anise seedpod oil

The angiotensin converting enzyme (ACE) is an important therapeutic target in the treatment of cardiovascular disease. Numerous ACE inhibitors are available clinically, and these are generally effective in treating hypertension. An increasing body of evidence suggests that oxidative stress, which results in an excessive generation of reactive oxygen species, has a key role in the pathogenesis of hypertension. Therefore, ACE inhibitors having antioxidant property are considered beneficial for the treatment of hypertension [28]. ACE inhibitors from medicinal plants might have less side effects than synthetic ones.

CSAO oil shows a similar *in vitro* antihypertensive activity (IC_{50} of 5.8 µg/mL) with trans-anethole (IC_{50} of 4.2 µg/mL) (Figure 5), being relatively abundant in CSAO (up to ~15 %), and captopril (IC_{50} of 2.8 µg/mL), a synthetic ACE inhibitor used in the treatment of cardiovascular

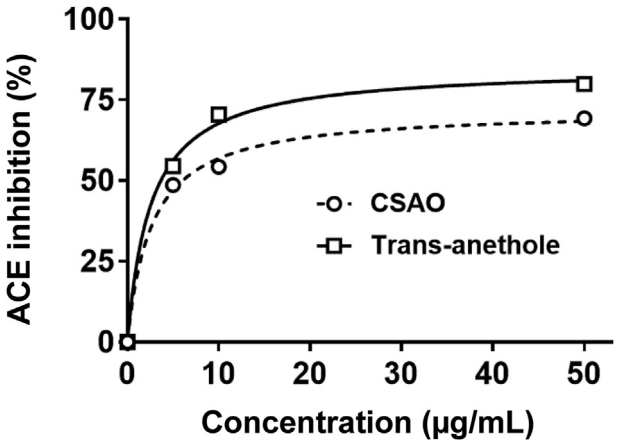

Figure 5. Antihypertensive activities of Chinese star anise whole seedpod oil (CSAO) and standard trans-anethole.

disease [29]. That said, minor components might also contribute in some way to the CSAO antihypertensive potency. These findings suggest that CSAO along with trans-anethole could be potential leads in the treatment of hypertension as natural ACE inhibitors.

CSAO oil exhibits moderate DPPH free radical scavenging, ferric ions reducing and anti-lipid peroxidation activities in a concentration-dependent manner with IC_{50} of 7.7 mg/mL, 6.5 mg/mL and 13.5 mg/mL, respectively (Figure 6A, B and C). Similar results were reported for star anise petroleum ether extract [30]. Trans-anethole however has poor antioxidant properties, as reported previously [31]. Thus, trans-anethole is not the principal compound that triggers CSAO antioxidant activity. Antioxidant properties of CSAO could be attributed to minor components, as suggested previously [32]. Therefore, in addition to its basic function as alternative lipid excipient with outstanding resistance to gastrointestinal lipolysis, the unconventional CSAO oil might have additional beneficial biological properties, i.e., antioxidant and antihypertensive activities, reinforcing its emerging role in designing new lipid formulations for the oral delivery of lipophilic drugs.

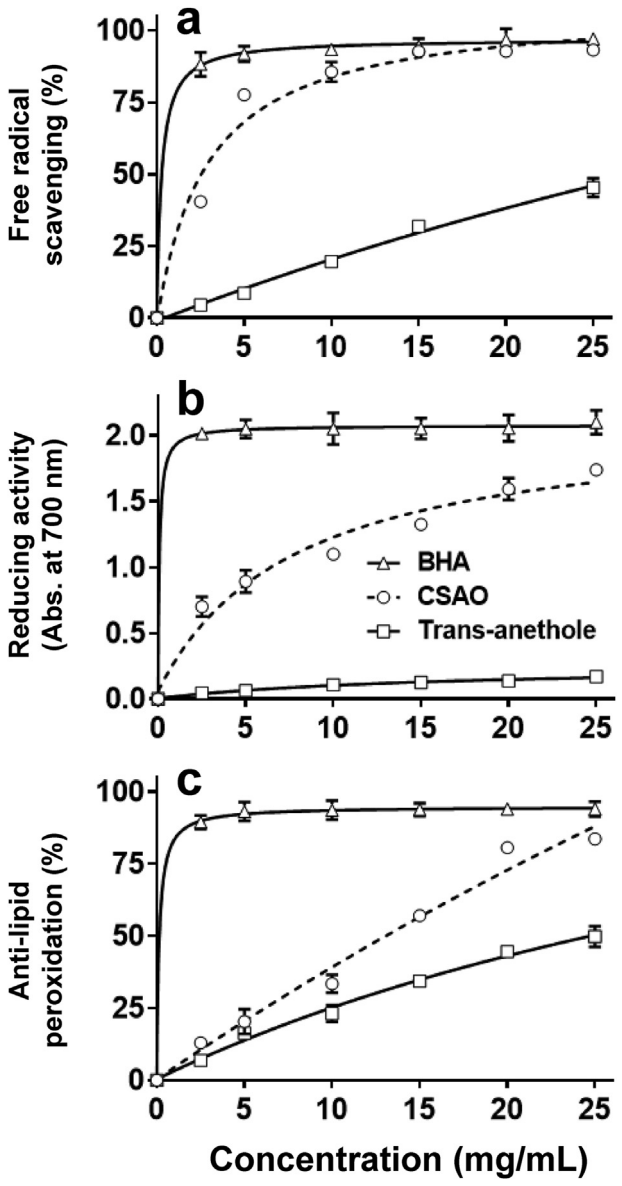

Figure 6. DPPH free radical scavenging activity (A), ferric ions reducing activity (B) and anti-lipid peroxidation activity (C) of Chinese star anise whole seedpod oil (CSAO), standard trans-anethole and BHA (positive control) at various concentrations.

To our knowledge, this is the first report that tentatively identified the unconventional star anise whole seedpod oil as a promising multifunctional excipient for the development of new lipid-based drug delivery systems. However, the fact that *in vitro* gastrointestinal lipolysis assays were performed without any poorly water-soluble drug will have to be considered in further studies to elaborate *in vitro* data as predictive as possible of the *in vivo* digestibility of lipid excipients. This said, *in vitro* formulation performance criteria require careful interpretation for understanding of *in vivo* performance in the complex and dynamic environment of the gut.

3. Materials and methods

3.1. Oil extraction

Dried Chinese star anise was purchased from a local market (Sfax, Tunisia) and finely powdered using a grinder. Dried star anise powder was extracted using maceration technique in hexane (1:5 w/v ratio) at room temperature. After filtration of the mixture by a fritted glass, the solvent was removed under *vacuum* using a rotary evaporator at 35 °C. The obtained oil was then stored in the dark at 4 °C for subsequent analyses.

3.2. TLC analysis

Neutral and polar lipids from Chinese star anise whole seedpod oil (CSAO) were analyzed by performing thin-layer chromatography (TLC) using aluminum sheets coated with 0.2 mm silica gel 60. For neutral and polar lipids analysis, chromatographic solvents containing diethylether: acetic acid (55:45:1) and chloroform:methanol:water (65:35:5) [33] were used, respectively. Iodine vapor was applied for developing TLC Plates.

3.3. GC-MS analysis

Fatty acid methyl esters, prepared as previously described [34], were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GC-2010 gas chromatograph. The fused fatty acid methyl esters column (30 m × 0.25 mm id, film thickness 0.25 μm; Supelco, USA) was coupled to a Shimadzu QP 2010 Plus mass-spectrometer (Shimadzu, Japan). The oven temperature was programmed at 50 °C for 1 min, then 25 °C/min to 200 °C, then 3 °C/min to 230 °C, and then left at 230 °C for 21 min. The injection port temperature was 250 °C and that of the detector was 220 °C (split ratio 1/5). Helium was the carrier gas with a flow rate of 1.2 ml/min. Mass spectrometer parameters were set at 70 eV ionization voltage, 200 °C ion source temperature and spectra acquisition over the 35–500 m/z mass range. Compounds were identified based on the Wiley registry of mass spectral data.

3.4. Triacylglycerols analysis

The analysis of CSAO triacylglycerols (TAGs) was performed according to the official chromatographic method of the Equivalent Carbon Number (ECN) which is formulated as ECN = CN - 2n; where CN is the number of carbon atoms of the fatty acid of the TAG and n is the number of double bonds of the TAG molecule [35]. The TAGs profile was obtained by a chromatographic system (HP1100, Agilent Technology, Germany) equipped with a differential refractometer detector and a Spherisorb analytical column (250 mm × 4.6 mm, Supelco, USA). The separation was conducted by an isocratic elution with acetone/acetonitrile mixture (60:40) at a flow rate of 1.5 mL/min and an injection volume of 20 μL of the oil dissolved in acetone. TAGs were identified based on standard TAGs.

3.5. Polyphenols, tocopherols and pigments content

CSAO total polyphenols were extracted by methanol/water (60:40). After centrifugation, total polyphenols from the methanolic phase were quantified using Folin-Ciocalteu reagent [36] and expressed as gallic acid equivalence (GAE). Chlorophylls and carotenoids were quantified by spectrophotometry [37]. α- and β-tocopherol levels were determined by HPLC method [38].

3.6. Acidity, iodine value and refractive index determination

Acidity, iodine value and refractive index of CSAO were determined according to International Organization for Standardization (ISO).

3.7. Spectroscopic analysis

UV–Vis absorption profiles of CSAO and standard trans-anethole, dissolved in hexane and methanol, respectively, were obtained through a PowerWave™ 200 scanning microplate spectrophotometer in conjunction with KC4 software (BIO-Tek Instruments, USA). The UV-Vis absorption spectra were recorded from 230 to 700 nm.

IR data were acquired with a Nicolet™ iS™ 10 FTIR Spectrometer equipped with a DTGS detector (Thermo Scientific, USA). IR spectra were recorded at 20 °C with 256 interferograms at 4 cm^{-1} resolution each and then Fourier transformed. During data acquisition, the spectrophotometer was continuously purged with dry filtered air (Balston regenerating desiccant dryer, model 75-45). For IR measurements, CSAO and standard trans-anethole were deposited on CaF_2 windows.

3.8. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was performed by a SETSYS Evolution instrument (Setaram, France). The sample was heated over the temperature range of 30–600 °C at a rate of 10 °C/min under an oxygen purge (10 mL/min) to establish a suitable environment for the oxidation process [39].

3.9. Lipase activity assay

Enzymatic activity was assayed by measuring the amount of free fatty acids (FFAs) released from a mechanically stirred oil/gum arabic emulsion using 0.1 M NaOH with a pH-stat (Metrohm 718 STAT Titrino, Switzerland) at a constant pH value of 8.5 and at 37 °C [40]. 10% w/v oil emulsions in 10% w/v gum arabic were prepared using a warring blender. The reaction mixture (15 mL final volume) was prepared by mixing 5 mL of the 10% w/v oil emulsion with 10 mL of the lipase assay buffer. The pancreatic lipase activity was assayed in the presence of 4 mM sodium taurodeoxycholate (NaTDC), 1 mM Tris, 150 mM NaCl, 3 mM $CaCl_2$ and a molar excess of colipase [40]. Novozyme® 435 activity was assayed in the presence of 1 mM Tris, 150 mM NaCl and 3 mM $CaCl_2$ [41]. The amount of enzyme used in assays corresponds to 10 units measured on olive oil emulsion. One lipase international unit corresponds to one μmol of FFAs released per minute.

3.10. Antioxydant and antihypertensive assays

Antioxydant activities of CSAO oil were performed spectrophotometrically by measuring linoleic acid/β-carotene bleaching activity [42], 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical scavenging activity [43] and ferric reducing power [44] as described previously. BHA was used as a reference standard. The angiotensin converting enzyme (ACE) inhibitory activity was carried out by measuring the hydrolysis rate of hippuryl-L-histidyl-L-leucine (Hip-His-Leu) as substrate using the spectrophotometric method [45]. IC_{50} values denote the half maximal inhibitory sample concentration.

Declarations

Author contribution statement

Ahmed Aloulou: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Jannet Kamoun: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Fatma Krichen, Imed Koubaa, Nacim Zouari, Ali Bougatef, Abdelkarim Abousalham: Contributed reagents, materials, analysis tools or data.

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Data availability statement

No data was used for the research described in the article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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