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Chemical characterization and biological activities of *Simmondsia chinensis* (Link) C. K. Schneid seeds oil

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Abstract: The chemical composition, main physicochemical properties, and biological activities of *Simmondsia chinensis* (*S. chinensis*) seeds oil were studied. The results revealed that the physiochemical characteristics of *S. chinensis* seeds oil were as follows: acid values 1.15 mg KOH/g, peroxide values 8.00 meq O_2 Kg^{-1}, iodine values 80.00 g/100 g of oil and saponification values 92.00 mg KOH/g, phenolic content 50.91 mg gallic acid equivalents/g extract. Gas chromatography analysis indicated that eicosenoic (55.50 %), erucic (20.43 %) and oleic (19.01 %) acids were the most abundant, saturated and unsaturated, fatty acids in the oil. Moreover, the evaluation of their antioxidant (DPPH, TAC), antibacterial, antidiabetic and acetylcholinesterase evinced interesting results. Seeds of *S. chinensis* constitute a substitute source for stable vegetable oil and protein with regard to nutritional and industrial applications.

Key words: *Simmondsia chinensis*; Seeds oil; Chemical composition; Physiochemical proprieties; Biological activities.

Introduction

The increasing interest in phytochemicals lies in supplying natural antioxidants used for food preservation, cosmetics and health care since they are environmentally more friendly and safer for consumption than their synthetic equivalents (1-3). Plants are known for their health protective effect owing to their phenolic components, charcterized by strong antioxidant activity against the reactive oxygen species (4-6).

Plants have a plethora of free radical scavenging molecules, namely phenolic compounds (phenolic acids, flavonoids), vitamins (C and E), and other endogenous metabolites (7-9). It is suggested that phytosterols are likely endowed with anti-inflammatory activities and antioxidant characteristics (10). These natural phytochemicals are spread in different plants parts, mainly in seeds (11).

S. chinensis is a dioecious plant, growing in desert and semi-desert areas. Thanks to its high economic value, the cultivation of *S. chinensis* has been implanted in a large number of countries. Besides its wide use for medicinal reasons, *S. chinensis* is employed as a traditional remedy for cancer, cold, dysuria, obesity, parturition, sore throat warts and wounds (12, 13). *S. chinensis* is also notorious for its seeds oil (liquid wax esters) which is mainly composed of straight chain monoesters in the range of C40-C44 (14). As far as cosmetics are concerned, *S. chinensis* oil incorporates a set of skin care products, mainly as a moisturizer, hair conditioner and a lubricant (15). Based on research studies, it is suggested that the seeds oil possesses an anti-inflammatory effect (16) and an antioxidant feature (17) and it promotes wound healing proprieties.

Bearing a high level of wax and proteins, these seeds represent a valuable raw material for various industries such as the *S. chinensis* wax producers and the animal food manufacturers, respectively (18). To our knowledge the chemical parameters and some biological proprieties of *S. chinensis* seeds were not investigated.

Materials and Methods

Simmondsia chinensis Seeds

The seeds of *S. chinensis* were collected from Sidi Bouzid, Tunisia, in December 2014 and authenticated by Prof. Mohamed Chaieb, Department of Biology. A voucher specimen (Number LCSN 132) was deposited in the Herbarium of the Laboratory of Organic Chemistry, Faculty of Science, Sfax University, Tunisia.

Extraction of seeds oil

An amount of 400 g of mature seeds of *S. chinensis* was ground into a powder and then macerated three times with 1 L of hexane for 24 h at room temperature. The received mixture was filtered through a filter paper (Whatman no.4) and concentrated further in vacuo at 40 °C.

Physicochemical properties of Seeds oil

Acid, iodine, saponification and peroxide values were determined with reference to respective standards (ISO 660. 1996) (19), (ISO 3961. 1996) (20), (ISO3657. 2002) (21) and (ISO 3960. 2001) (22).

Fatty acid composition

Fatty acid analysis was performed after completing alkaline treatment obtained by dissolving the oil (0.05 g) in hexane (1 mL) and adding a solution of potassium hydroxide (1 mL; 2N) in methanol (23). The analysis of fatty acid was guaranteed by gas chromatography via a Shimadzu 17A gas chromatograph equipped with a flame ionization detector (FID) and a capillary column. The operation conditions were as follows: the column temperature was programmed from 180 to 240°C at 5°C/min and the injector and detector temperatures were set at 250°C; nitrogen was the carrier with a flow of 1 mL/min. The chromatographic separation was accomplished by injecting 1 µL of solution into a capillary column characterized by a 30 m length, a 0.32 mm diameter, and a 0.25 µm film thickness. The polar stationary phase was cyanopropylmethyl/phenylmethylpolysiloxane (1:1, v/v). The identification of peaks was determined via a comparison between their retention times and those of authentic reference compounds. In order to express the fatty acid composition, the yield of each fatty acid in the lipid fraction was adopted.

^{1}H-NMR profile

^{1}H-NMR spectra were obtained using a Bruker Ascend 400 NMR Spectrometer operating at 400 MHz in $CDCl_3$. 20 mg of seeds oil were dissolved in $CDCl_3$ (1 mL) in an NMR tube and readings were taken between 0–14 ppm. Coupling constants are given in Hertz. The chemical shifts are expressed in δ (ppm) (24).

Total phenolic content (TPC)

Singleton's method, slightly modified by Oktay, was adopted to determine oil phenolics concentration (25). In the present analysis, the seeds methanolic solution was realized at a concentration of 1 mg/mL. In fact, one milliliter of the last solution was mixed with 0.5 mL of Folin-Ciocalteu reagent and 0.5 mL of Na_2CO_3 solution (2%). 3 mL of water were added were added to the resulted mixture. Together, a blank was prepared. After that, all the samples were incubated for 90 min in the dark. A spectrometer at a wave length of λmax = 760 nm was used to measure absorbance. Each analysis was repeated in triplicate after which a mean value of absorbance was recorded.

Total antioxidant capacity (TAC)

The method of Prieto was employed to determine the total antioxidant activity of seeds oil (26). Briefly, a combination of 0.1 mL of sample at different concentrations (0.0625, 0.125, 0.25, 0.5 and 1 mg/mL) with 1 mL of reagent solution was added (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction solution was incubated at 95°C for 90 min in water bath. Then, the absorbance of all the sample mixtures was measured at 695 nm. Total antioxidant activity is expressed in mg vitamin E equivalents per gram of extract (mg antioxidant/g extract).

DPPH radical scavenging assay

The oil antioxidant activity was estimated by 2,2′-diphenyl picrylhydrazyl (DPPH) assay with a minor modification (27). Briefly, different sample concentrations (0.0625, 0.125, 0.25, 0.5 and 1 mg/mL) and 0.1 mM DPPH radical solution were prepared in methanol. 2 mL of a DPPH methanolic solution was added to a 1 mL of either methanolic solution of extract (sample) or methanol (control). The mixtures were vortexed for 1 min and then left to stand in the dark at room temperature. After 30 min absorbance was observed at 517 nm. The percentage of inhibition (PI %) was calculated using the following equation:

$$PI\ (\%) = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

$A_{control:}$ Absorbance of the methanol control; $A_{sample:}$ Absorbance of the extract.

Synthetic antioxidant, BHT, was used as a positive control. The preparation of bleached DPPH solution was conducted by adding 2 mL of 0.1 mM DPPH solution to 1 mL of BHT. DPPH radical–scavenging activity was calculated as the concentration that scavenges 50% of DPPH free radical and it has, thus, RSA = 50% (IC_{50}) (28).

Determination of antibacterial activity

The seeds oil antibacterial activity was accomplished by means of the agar well-diffusion method (29). Consequently, each of the sterile Wattman paper disks N° 3 and of diameter 6 mm is impregnated with 20 µL of seeds oil at a concentration of 50 mg / mL and placed on the middle of the petri dish in presence of disks impregnated with aqueous solution (negative controls). Disks of ampicillin were marketed (at 10 µg / disc) as positive controls. Subsequently, the incubation of the dishes was performed for 2 h at 4 °C and then at 37 °C for 24 h. The inhibition zones diameters surrounding the discs incorporating the samples to be tested were measured.

Acetylcholinesterase enzyme inhibitory activity (AChE)

The acetylcholinesterase test was examined using micro-plate assays. The enzyme activity was measured by observing the increase of a yellow color generating from the reaction of thiocholine with the dithiobisnitrobenzoate ion. To measure AChE activity, the assay described by Ellman (30, 31) was used. Furthermore, 125 µL of DTNB(3 mM), 50 µL of sodium phosphate buffer (pH 8.0) and 25 µL of sample dissolved in DMSO then 25 µL of 0.5 U/mL AChE were added in a 96-well microplate and incubated for 15 min at 25 °C. Afterwards, the initiation of the reaction was conducted by adding 25 µL of acetylthiocholine iodide (ATCI). After the acetylthiocholine iodide hydrolysis, a yellow 5-thio-2-nitrobenzoate anion was formed owing to the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 405 nm. The yield of inhibition (PI) was calculated with reference to the equation that follows:

$$PI\ (\%) = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

Where Acontrol is the absorbance of the control and Asample is the absorbance of the test sample. Ta-

crine was used as positive control. The IC50 was calculated by log-probit analysis.

In vitro α-amylase inhibition assay

The *S. chinensis seeds* oil α-amylase inhibition was determined with respect to the spectrophotometric assay, using acarbose as the reference compound (32). The sample was dissolved in DMSO to the different obtained concentrations of 50, 100 and 200 µg/mL. The enzyme α-amylase solution was prepared by mixing 3.246 mg of α -amylase (EC 3.2.1.1) dissolved in 100 mL of phosphate buffer, (40 mM, pH 6.9). Then, positive control (acarbose) was prepared. The absorbance was measured at 405 nm and control reaction was carried out without the *S. chinensis* oil. The following equation was used to calculate the percentage inhibition:

$$PI\ (\%) = [\frac{A_{control} - A_{sample}}{A_{control}}] \times 100$$

$A_{control:}$ Absorbance of the methanol control; $A_{sample:}$ Absorbance of the extract.

Results

The *S. chinensis* seeds oil extraction yielded a yellow color. The taste and odor were varied with the oil's composition, the specific method of production and the state of rancidity. Table 1 reports the result of physicochemical characteristics of *S. chinensis* seeds oil.

Relying on the acid value (1.15 mg KOH/g), the *S. chinensis* seeds oil could be recommended for industrial use. The high peroxide yield of *S. chinensis* seeds oil (8 meq O_2/Kg of oil) indicated the richness on hydroperoxide, which can be attributed to the increasing level of unsaturated fatty acids (Table 1) (33). Saponification value is a sign of the average molecular weight and thus the chain length. The low saponification value of the examined oil (92.00 mg KOH/g) suggests that the mean molecular weight of fatty acids is lower or that the number of ester bonds is fewer. The high iodine value (80.00 g/100 g) is ascribed to the high percentage of unsaturated bonds number (34).

Fatty acids composition

As a parameter for oil classification and in order to depict and to determine adulteration, the fatty acid composition was used. This composition has a straight relationship with the oil quality (Table 2). The results evinced the principal fatty acid components to be eicosenoic (55.50 %), erucic (20.43 %) and oleic (19.01 %) acids in decreasing order, while docosenoic and palmitic acids were minor in *S. chinensis* seeds oil. The yields of saturated and unsaturated fatty acids of the *S. chinensis* seeds oil were 3 % and 97 %, respectively.

Table 1. Physicochemical properties of *S. chinensis* seeds oil.

Parameter	*S. chinensis* oil
Chemical composition of the seeds	
Yields (%)	6.35 ± 0.20
Acid value (mg KOH/g of oil)	1.15 ± 0.10
Peroxide value (meq O2/Kg of oil)	8.00 ± 0.10
Saponification value (mg KOH/g of oil)	92.00 ± 0.20
Unsaponifiable matter (% of oil)	50.80 ± 0.10
Iodine value (g/100 g of oil)	80.00 ± 0.22
Color	Yellow

Table 2. Fatty acids composition (%) of *S. chinensis* seeds oil.

Fatty acid	Carbon Length	Retention time (min)	Seeds oil (%)
Palmitic	C16:0	3.60	2.02
Oleic	C18:1	5.80	19.01
Eicosenoic	C20:1	6.23	55.50
Erucic	C22:1 ω9	10.20	20.43
Docosenoic	C22:1ω11	11.07	3.03
SAFA			3.60
MUFA			96.30

SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids.

Table 3. Chemical shift assignments of corresponding groups of *S. chinensis* seeds oil from ^{1}H-NMR spectrum.

Signal	Chemical shift (ppm)	Functional group
1	0.83-0.93	Terminal methyl groups
2	1.22-1.40	Methylenes, except the lowing
3	1.56-1.67	Methylenes in β to the Carbonyl groups
4	1.98-2.10	Methylene protons of the allylic groups
5	2.29-2.39	Methylenes in α to the carbonyl groups
6	2.76-2.81	Diallyl methylenes
7	4.13-4.32	Glyceryl methylenes
8	5.25-5.30	Glyceryl methines
9	5.30-5.42	Olein protons

^{1}H-NMR Profile

We used ^{1}H-NMR spectroscopy in order to characterize *S. chinensis* seeds oil and to collect further data about this oil. The relevant assignments of the chemical shifts in the ^{1}H-NMR spectrum of *S. chinensis* seeds oil are displayed in Table 3 and Figure 1. The main signals were observed as triplets at 0.88 and 0.97 ppm related to terminal methyl groups, multiplets at 1.27 and 1.31 ppm due to the methylene protons of carbon chain, multiplet centred at 1.63 ppm assigned to β-carbonyl methylene protons, multiplet at 2.09 ppm pertaining to methylene protons of allylic groups, triplet centred at 2.31 ppm corresponding to methylenes in α to carbonyl groups, another triplet at 2.81 ppm distinguishing diallyl methylene protons, two double doublets centred at 4.16 and 4.31 ppm related to glyceryl methylenes, multiplet at 5.34 ppm overlapped with another one at 5.38

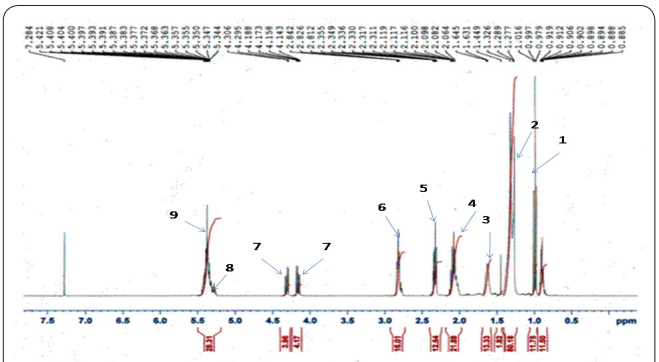

Figure 1. ^{1}H-NMR spectrum of *S. chinensis* seeds oil 400 MHz in $CDCl_3$.

Table 4. Phenolic content, total antioxidant capacity (TAC), DPPH radical-scavenging assay acetylcholinesterase inhibitory and Alpha-amylase activities of *S. chinensis* seeds oil.

	TPC mg GAE/g of oil	TAC (mg antioxydant/g of oil)	DPPH IC_{50} (µg/mL)	%AChE Inhibition	α-amylase IC_{50} (µg/mL)
Oil	50.91 ± 0.10	94.74 ± 0.20	215 ± 0.15	60.00 ± 0.11	20.20 ± 0.22
BHT	-	-	26.00 ± 0.08	-	-
Vitamine E	-	470 ± 1.50	17.00 ± 0.13	-	-
Tacrine	-	-	-	80.5 ± 0.10	-
Acarbose	-	-	-	-	14.88± 0.10

ppm related to glyceryl methines and to oleinic protons, respectively.

Total phenol content

The total phenol content (TPC) was determined using the Folin Ciocalteu reagent and the results were expressed in milligram of gallic acid equivalents per gram of extract. The phenolic value is illustrated in Table 4. The oil showed a high amount of phenolic compound, 50.91mgGAE/g of oil. Owing to their impact on sensory features, the shelf life of oil polyphenols displays a significant technological value.

Total antioxidant capacity

The evaluation of the antioxidant capacity of *S. chinensis* seeds oil was conducted by the phosphomolybdate method, based on the reduction of Mo^{6+} to Mo^{5+} by the sample analyte and the subsequent formation of green phosphate/ Mo^{5+} compound with a maximum absorption at 695 nm. Table 4 indicates that *S. chinensis* seeds oil revealed a considerable antioxidant activity which is consistent with a high absorbance value, suggesting that the sample possesses significant antioxidant activity. The antioxidant capacity value of *S. chinensis* seeds oil was 94.74 mg antioxidant/g extract. This result is completely proportional with the amount of polyphenols.

DPPH radical scavenging assay

The antioxidant activity of the obtained oil was evaluated by the DPPH-H radical-scavenging assay. Hydrogen-donating antioxidant presence can be observed as a decrease in terms of DPPH solution absorbance. The concentration of antioxidant, needed to decrease the initial DPPH· concentration by 50% (IC_{50}), is a broadly employed parameter to measure the antioxidant activity. The obtained result from this test was illustrated in Table 4. The *S. chinensis* seeds oil was able to decrease the stable radical DPPH to the yellow colored DPPH-H with IC_{50} value *S. chinensis* (215.00 ± 0.15 µg/ mL). A high antioxidant activity of BHT and vitamin E was detected with IC_{50} values of 26.00 ± 0.08 µg/ mL and 17.00 ± 0.13 µg/ mL, respectively. The radical scavenging activity of this oil could be related to α-tocopherols and phenolic compounds.

Acetylcholinesterase enzyme inhibitory activity

Acetylcholinesterase inhibition is an important drug treatment strategy against Alzheimer's disease and, recently, great attention has been paid to find naturally acetylcholinesterase inhibitors to replace synthetic drugs, viz tacrine and donepzil (having some adverse effects) (35)**.**

The result related to acetycholinesterase (AChE) inhibitory activity of *S. chinensis* seeds oil is given in Table 4. The *S. chinensis* seeds oil showed to be able to inhibit 62.0 % of AChE activity. Tacrine used as a standard has a strong inhibition of acetylcholinesterase (80.5 %). According to Vinutha (36) AChE inhibition value higher than 50 % indicates potent inhibition.

***In vitro* α-amylase inhibition assay**

Several synthetic drugs are used as potent inhibitors of α-amylase and lipase in the intestine. In this respect, natural α-amylase inhibitors are beneficial in reducing post-prandial hyperglycemia by delaying the digestion of carbohydrates and, consequently, the absorption of glucose. This study investigated the inhibition potential of *S. chinensis* seeds oil on pancreatic α-amylase activity. As illustrated in Table 4, the IC_{50} value of *S. chinensis* oil was (20.20 µg/mL), indicating its interesting inhibitory activity against the pancreatic α-amylase in comparison with that of the pure standard acarbose (IC_{50} = 14.88 µg/mL).

Antibacterial activity

Antibacterial activity of *S. chinensis* seeds oil was evaluated against six pathogenic strains namely *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus subtilis*, *Salmonella Sp* and *Salmonella enteric*. The obtained results were summarized in Table 5. *S. chinensis* seeds oil displayed varied antibacterial activities against both, Gram (+) and Gram (-) bacterias.

S. chinensis oil inhibited the growth of six bacterial strains with a zone diameter of inhibition ranging from 17.0± 1.0 mm with *S. chinensis* seeds oil against *S. enteric*, to 13.0± 0.2 mm with *S. chinensis* oil against *B. cereus*. *S. chinensis* seeds oil showed a higher inhibition activity across the studied pathogens and it appeared more active than penicillin tested concentration.

Table 5. Antibacterial activity of *S. chinensis* seeds oil using agar disc diffusion [(a)].

Strains	*Enterococcus faecalis*	*Listeria monocytogenes*	*Bacillus cereus*	*Bacillus subtilis*	*Salmonella Sp*	*Salmonella enterica*
Oil	10.5± 0.1	12.0± 0.2	13.0± 0.1	23.0± 0.5	11.0± 1.0	17.0± 0.1
Penicillin	14.0± 0.1	19.1± 0.2	13.1± 0.1	22.0± 0.2	15.0± 0.1	20.2± 0.1

[(a)]Values expressed are means ± SD of three parallel measurements ($p<0.05$).

Discussion

S. chinensis seeds oil is considered as a potential asset for human nutrition, especially as it exhibited a low amount of saturated fatty acids (3%) that are involved in cardiovascular diseases. Interest in PUFA as health promoting nutrients has significantly expanded in recent years (37). Furthermore, a good agreement was noticed between each of the iodine value of the *S. chinensis* seeds oil composition and the proton NMR analysis.

Using *S. chinensis* seeds oil as a natural antioxidant agent could have beneficial effects pertaining to alleviating chronic diseases' risks, namely cancer and cardiovascular disease and this oil could also be useful for the food drug industry (38).

The antioxidant results were in accordance with *S. chinensis* data conducted in other areas of the world (39). This can be explained by the fact that phenolic composite, phytosterols and α-tocopherol were constituents of *S. chinensis*, and these composites have an antioxidant activity, as previously proposed by Evans (40). *S. chinensis* also has a group of nitrile glycosides known as simmondsin. These pharmacologically active substances probably have a role in the antioxidant activity as reported by Abdel-Wahhab (41). These composites, including flavonoids and phenolic acids, are notably responsible for antioxidant capacities with higher phenolic contents (42).

The antibacterial activity appears to correlate well with the total phenolic value of *S. chinensis* seeds oil.

In addition to being widely recognized, the antimicrobial action of phenolics is associated to their ability of denaturizing proteins. They caused the leakage of cytoplasmic constituents such as proteins or minerals and testifyed their ability to check the cells wall. Phenolics, also known to bind to the peptidogly, can lead to the breaking of the bacterial cell-wall integrity. Recently, it has been claimed that these compounds possess anti-Alzheimer properties (43). *S. chinensis* has long been used for their excellent medicines proprieties against oxidative stress and diabetic (44). It has been investigated in many plant species that the phenolic constituents could significantly contribute to the antioxidant capacity of these species. Therefore, the higher amount of phenolics in *S. chinensis* can be taken as a good sign of its higher antioxidant capacity. These results revealed that *S. chinensis* may be suggested as a potential source of natural phenolic compounds, endowed with several activities especially antioxidant activity.

It is worthwhile to mention that acarbose was used for the management of post-prandial hyperglycemia, however, reportedly, this agent was related to several health side effects (45). The appreciable α-amylase inhibitory action of *S. chinensis* seeds oil could be related to its phenolic compounds content.

The present study on the chemical composition, physiochemical properties, antioxidant, antidiabetic, antibacterial and acetylcholinesterase activities of *S. chinensis* seeds oil indicates that the seeds could be considered as a potential substitute for oil. The seeds contain all essential fatty acids, notably eicosenoic, erucic and oleic acids. However, these three unsaturated fatty acids, beneficial for the human body, are highly recommended in vegetable oils. The utilization of *S. chinensis* seeds oil from Tunisia could be performed in a wide range of fields, viz. nutrition, cosmetics and pharmaceutical products.

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Conflicts of Interest

Non conflict of interest declared

Abbreviations

AChE, acetylcholinesterase enzyme; BHT, butylated hydroxytoluene; TAC, total antioxidant capacity; TPC, Total phenolic content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ^{1}H-NMR, proton nuclear magnetic resonance; MUFA, monounsaturated fatty acids; ω, omega; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids.

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