Chemical and Fatty Acid Compositions of Crude and Purified Extracts Obtained from *Datura innoxia* Seeds Extracted with Different Solvents

Sadok Mokbli¹, Hassen Mohamed Sbihi²*, Imededdine Arbi Nehdi², Mohammad Azam², Ayari Fadhila³, Moufida Romdhani-Younes⁴, and Saud Ibrahim Al-Resayes²

¹ Superior Institute of Biotechnology of Sidi Thabet, Sidi Thabet 2020, TUNISIA
² King Saud University, College of Science, Chemistry Department, P.O. BOX 2454, Riyadh 1145, SAUDI ARABIA
³ Carthage University, Faculty of Sciences of Bizerte, Zarzouna 7021, TUNISIA
⁴ Faculty of Sciences of Tunis, Department of Chemistry, Laboratory of Structural Organic Chemistry, 2002 University of Tunis El Manar Tunis, TUNISIA

Abstract: Oils play a key role as raw materials in a variety of industries. The aim of this study was to evaluate the potential of *Datura innoxia* seed oil cultivated in Saudi Arabia for industrial purpose and to study the effects of hexane, chloroform, and isopropanol as extraction solvents on the compositions of the extracts. The results showed that the hexane and chloroform extracts were mainly neutral oils which were rich in linoleic (≈46%) and oleic (≈31%) acids. However, the isopropanol extract contained large amount of neutral oil and organic acids. Neutral oil contained mainly palmitic acid (40.2%) and some important and valuable epoxy (15.4%) and cyclopropane (13.2%) fatty acids. Analysis of the sterol and tocopherol levels of the crude and purified oil extracted revealed that they were significantly affected by the extraction solvent used.

Key words: *Datura innoxia* seeds, extraction solvent, seed oil, unusual fatty acids, tocopherols, sterols

1 Introduction

In recent years, there has been increasing interest in the manufacture and use of non-edible and edible oils. They are an important subject in a wide range of scientific and industrial disciplines. For instance, non-edible and edible oils are widely used in the production of biofuels, especially biodiesel, and other non-food industrial uses¹.

To date, the demands for oils of both edible and non-edible oils purpose are ever increasing and conventional sources are enable to meet these demands owing to the increasing world population. The world market is largely dominated by palm, canola, coconut, and soybean oils. Furthermore, other vegetable oils are widely used for industrial applications including castor and Tung oils. In recent years, there has been an increasing interest in the use of unexploited plant species as a secondary source of oils. Many of them contain large quantities of oils and a high proportion of industrially fatty acids. Moreover, no oil from any source has been found to be suitable for all purposes since oil from different sources has different range of chemical compositions².

Several studies have demonstrated that non-edible and edible oils are rich sources of valuable lipophilic compounds such as sterols, squalene, tocopherols, carotenoids, and essential fatty acids¹. Furthermore, polyunsaturated fatty acids are in high demand in cosmetic sectors. These essential fatty acids are required for skin treatment and hair growth.

Shahidi² reported that four types of process have been generally used to extract oil from fruits or seeds: hydraulic press, expeller or screen press extraction, prepress solvent extraction, and direct solvent extraction. These oil extraction processes have no effect on fatty acid composition, whereas the solvent extraction process has the benefit of significantly higher oil yield compared to mechanical press processes. In addition, Akoh³ showed that solvent extraction process gave 11.5% more oil yield than the screw press method did, and less oil remained in the meal. Selection of the oil extraction process is related to the yield of the starting materials. When the oil yield is higher than
30%, prepress solvent extraction is used to produce oil. In contrast, when the oil yield is less than 30%, the started materials is extracted by direct solvent extraction.

It is well established that, in the development of oilseed extraction technologies, the choice of solvent is crucial in determining the type and quantity of the final oil, which will typically contain triacylglycerols and different amounts of valuable lipid components, such as sterols, tocopherols, pigments and vitamins components\(^4\),\(^5\).

The selection of solvent is usually dependent upon the availability, operation safety, extraction efficiency, product quality, and cost. Recently, toxicity, bio-renewability, and environmental friendliness has been added to the solvent selection criteria\(^6\). Among these solvents are alcohols and hydrocarbons. Many halogenated solvents have been used in the past and are effective in extracting edible oil. However, these solvents have various degree of toxicity and therefore, are not likely to be used for edible oil extraction. Lipids with various functional group of low polarity are very soluble in hydrocarbon solvents like n-hexane and chloroform. n-Hexane has a low boiling point, which facilitates the recovery of solutes and solvent recycling. Chloroform is a power and excellent solvent power and it has outstanding physicochemical properties, low inflammability and high volatility\(^7\).

Short-chain alcohols, such as isopropanol, are widely regarded because of their low toxicities and superior operational safety\(^8\). Additionally, these alcohols are able to extract greater amounts of unsaponifiable material owing to their high polarity\(^9\). Moreover, Goncalves et al.\(^7\) showed that short-chain alcohols are used in deacidification of edible oils by solvent extraction method. The use of these alcohols increases the efficient of extraction of free fatty acids and reduces the neutral oil loss.

The effects of the type of extraction solvent used on the oil yield and natural product content of the extract have been widely investigated. Oladipo and Betiku\(^1\), Stevanato and da Silva\(^5\), Ibrahim et al.\(^9\), Castejón et al.\(^10\), and Bhutada et al.\(^11\) reported that the type of solvent affects the oil yield. In addition, Stevanato and da Silva\(^5\), Capellini et al.\(^7\), and Tir et al.\(^12\) concluded that the type of solvent affects the contents of tocopherols, tocotrienols, and sterols. Although there are many reports in the literature on the effect of the type of solvent used in extraction, most are focused on the fatty-acid and natural-product contents of the extract.

\(D.\ innoxia\), also called the thorn apple, is a species in the family Solanaceae. It is an annual herb distributed throughout most parts of the world. \(D.\ innoxia\) is a shrubby plant with a grayish appearance. Its flowers are white and trumpet-shaped, and its fruit is an egg-shaped spiny capsule. It splits open when ripe, dispersing the seeds within. Furthermore, \(D.\ innoxia\) is a very important medicinal plant as it is a well-known source of tropane alkaloids\(^2\). On the other hand, Naidoo\(^13\) reported that the high level of tropane alkaloids makes the consumption of \(Datura\) fruits by humans and animal not recommended.

It has been reported that \(Datura\) seeds give an acceptable oil yield. Moreover, \(D.\ innoxia\) seed oil contains the highest level of total tocopherols and sterols, compared to \(D.\ stramonium\), \(D.\ metel\) and \(D.\ tatula\)\(^14\). These components, sterols and tocopherols, are used to authenticate oils and detect adulteration\(^15\). Extraction of oils from \(D.\ innoxia\) seeds with different type of solvent with varying polarity may improve the level of natural products. Fatima et al.\(^15\) demonstrated that the pharmacological potential of medicinal plant was significantly improved by the polarity of solvent extraction of \(D.\ innoxia\) fruit.

To the best of the authors’ knowledge, no previous study has addressed the effects of extraction solvent on the diversity of fatty acids and other organic compounds in the specific extracts. There is little published data on the fatty-acid profiles and physicochemical properties of oils extracted from \(D.\ innoxia\) seeds. Furthermore, no previous study has investigated the fatty-acid, sterol and tocopherol compositions of crude and neutral oils obtained from \(D.\ innoxia\) seed or pod using extraction solvents other than hexane. Therefore, the main aim of this study was to investigate the effect of extraction solvent on the type and contents of fatty acids extracted from \(D.\ innoxia\) pods and seeds. The extracts were prepared by the Soxhlet method using hexane, chloroform, or isopropanol as solvents. Moreover, we determined the tocopherol and sterol contents of the different extracts of crude and purified oils. Thus, it is hoped that this study will enhance our knowledge of \(D.\ innoxia\) seed and pod oils extraction and its products, especially in terms of how different extraction solvents affect the composition of the crude and purified oils produced.

2 Materials and Methods

2.1 Materials

\(D.\ innoxia\) plants were collected (June 2019) from the Wadi Hanifa in the Nejd region of Riyadh Province, Saudi Arabia. Unambiguous identification of the plant material was performed by Dr. Jacob Thomas Pandalayil of the Biochemistry Department, Science College, King Saud University. Isopropanol, hexane, and chloroform (analytical grade) were purchased from Sigma-Aldrich. Internal standards for tocopherol and sterol analyses were also supplied by Sigma-Aldrich.

2.2 Oil extraction

The fruits were cut in two. Then, the seeds were removed and manually cleaned by removing the undersized material. Finally, the seeds were refined to remove the very
light materials. The seeds and pods were flaked using a universal mill (IKA-M20). The flaked material was placed in cellulose paper and then subjected to Soxhlet extraction in n-hexane, chloroform, or isopropanol. The solvent was stripped from the extract using rotary evaporation (R-210 BUCHI, Flawil, Switzerland); this was done first under atmospheric pressure and then, under vacuum. The weight of the extracts was determined to calculate the extract content. The result was expressed as the extract percentage in the dry seeds and pods. The percentage yield of extracts was calculated as follows:

\[
\text{Yield of extract (\%)} = \frac{\text{Weight of the extract (g)}}{\text{Total weight of the seeds (or pods) (g)}} \times 100
\]

The purified oil was obtained using liquid–liquid extraction. The isopropanol liquid extract was extracted with n-hexane (3 × 10 mL). The combined hexane layers were evaporated using a rotary evaporator at a temperature of 70°C and then under vacuum. After evaporation, the product (purified oil) was a colorless liquid. The final product mass was determined to calculate the percent yield of purified oil relative to the mass of the starting seed samples. The n-hexane and chloroform extracts were used as crude oils without any process of refining. The percentage yield of oils was calculated as follows:

\[
\text{Yield of oil content (\%)} = \frac{\text{Weight of crude (or purified) oil (g)}}{\text{Total weight of the seeds (or pods) (g)}} \times 100
\]

The non-hexane fraction is rich in organic acids. In order to identify their composition, the organic acids were converted into their corresponding esters according to the procedure used by Lam et al.\(^\text{10}\). The esters formed were then identified and quantified by gas chromatography-mass spectrometry (GC-MS).

The free fatty acid (as oleic) content was determined using the standard procedure (ISO 660:1996)\(^\text{17}\). The theoretical iodine value was calculated using the constants and the composition of unsaturated fatty acids as previously reported by Ham et al.\(^\text{18}\). The chlorophyll and carotenoid contents were determined according to the method described by Allalout et al.\(^\text{19}\).

### 2.3 Fatty-acid compositions

The fatty acid methyl esters (FAMEs) were prepared according to the procedure used by Mokblı et al.\(^\text{20}\). FAMEs were analyzed by GC-MS (QP2010 Ultra, Shimadzu, Japan). The FAMEs were separated using an Rxi-5Sil Ms column (30 m; 0.25 mm i.d.; 0.25 µm). Helium was employed as the carrier gas at a constant column flow of 1.5 mL/min. The GC oven temperature program was as follows: 115°C for 2 min, increased at 2°C/min to 240°C, and then maintained at this temperature for 35 min. The injector and detector temperatures were held at 225 and 275°C, respectively. The identification of the FAMEs was performed by MS running in scan mode at 70 eV and using electron impact ionization. The mass spectra thus obtained were compared with those in the NIST library database.

### 2.4 Tocopherol analysis

Tocopherols in different seed oils were analyzed by high-performance liquid chromatography (HPLC; LC-20AT pump, Shimadzu, Japan) according to standard ISO 9936\(^\text{21}\). A fluorescence detector was used with excitation and emission wavelengths of 294 and 330 nm, respectively. The flow rate of the mobile phase (hexane/isopropanol, 99.5:0.5, v/v) was 0.5 mL/min. Tocopherol isomers were separated on a Hypersil silica column (15 cm; 3.0 mm i.d.; 0.25 µm). The relative concentrations of the tocopherol isomers were determined by comparison with internal standards of known concentrations analyzed under the same conditions.

### 2.5 Sterol analysis

Sterol analysis was performed following the ISO 12228\(^\text{22}\) method with a slight modification. Briefly, a mixture of oil (0.25 g) and cholesterol (1 mg/mL in acetone) in an ethanolic solution of KOH (0.5 M) was heated at reflux for 30 min. After cooling, 100 mL of double-distilled water was added. The water phase was extracted with diethyl ether (3 × 10 mL). The combined organic layers were dried over MgSO\(_4\) and evaporated in vacuo. Purity was determined using an aluminum oxide column and thin-layer chromatography plates eluted with hexane/diethyl ether (1:2, v/v). The sterols were transformed into sterol trimethylsilyl ethers using a derivatization agent (N-methyl-N-(trimethylsilyl) trifluoroacetamide). The solution was heated for 30 min in an oven set at 105°C and, after cooling and adding 1 mL hexane, directly injected into the GC-MS apparatus, as described previously. The identification and relative concentrations of sterol isomers were determined by matching their mass spectra with those in the NIST library and by comparing their retention times and concentrations with those of similarly analyzed internal standards.

### 2.6 Statistical analysis

All data analyses were carried out using GraphPad Prism software, version 6. Comparisons between the groups were made using a one-way ANOVA (the Bonferroni test was used to compare all pairs of columns) at a 95% confidence level. Significance levels were set at the 5% level (p < 0.05). All measurements were carried out in triplicate.
3 Results and Discussion

The seeds of *D. innoxia* are flat, brown, and of a similar shape and size (Fig. 1). The mean weights of the pods and seeds are 5.1 ± 0.4 and 3.9 ± 0.3 g, respectively. The average weight percent of the seeds in a pod is 76.6 ± 3.2%. The average number of seeds in a pod and the average mass of 100 seeds are 399 ± 34 seeds and 0.98 ± 0.03 g, respectively. The seeds of *D. innoxia* contain 3.7 ± 0.2% moisture. These results are different from those of Gazizov et al., who reported that the average seed mass and moisture content were 1.2 g and 5.8%, respectively (Table 1).

The results obtained from GC-MS analysis of *D. innoxia* seed oils (DSOs) extracted with hexane (crude oil; DSOH), chloroform (crude oil; DSOC), or isopropanol (purified oil; DSOI) are presented in Table 2.

A significant difference for the fatty-acid profile of DSOI compared to those of DSOH and DSOC is immediately apparent. No significant differences (p > 0.05) are observed between DSOH and DSOC in terms of fatty acid type and content. Seventy fatty acids are identified, among which linoleic acid (C18:2Δ12,15c) is the most abundant (=46%), followed by oleic (C18:1Δ9c; ≈32%), palmitic (C16:0; = 15%), and stearic (C18:0; ≈3%) acids. Moreover, approximately 46.5% of the fatty acids are polyunsaturated, 33.5% are monounsaturated, and 20% are saturated. These results are in good agreement with those of other studies, in which linoleic, oleic, palmitic, and stearic acids were the major fatty acids identified in DSO. Ramadan et al. reported that DSO contains 52.8% linoleic, 28.4% oleic, 14.7% palmitic, and 2.34% stearic acids. Conversely, Gazizov found that DSO contains 40.9% linoleic, 37.9% oleic, 11.3% palmitic, and 3.7% stearic acids.

Fatty acid composition of DSOH and DSOC is almost close to soybean oil. Soybean oil contains mainly 8 to 13.3% of palmitic acid, 17.7 to 26.1% of oleic acid, 49.8 to 57.1% of linoleic acid, and 5.5 to 9.5% of linolenic acid. *D. innoxia* oil’s stability is better than soybean oil’s stability due to its low content of linolenic acid (≈0.45%). *D. innoxia* neutral oil contains a high level of both oleic and linolenic acids. Oleic acid is the main fatty acid present in olive oil and has been reported that oleic acid plays an important role in cancer prevention, coronary disease, and hypertension. Moreover, liver was enable to synthesize enough oleic acid to ensure a normal composition of cellular membrane. Linoleic acid is an essential fatty acid and cannot be synthesized by the body and thereby it is considered as essential in the diet. For industrial use, Mackeon et al. reported that the feedstock for polymer preparation is from vegetable oils enriched in C16-C18 saturated, monounsaturated and diunsaturated fatty acids. Epoxidized oils can be used in paints, plasticizers, adhesives, and coatings. Soybean oil is used as feedstock to synthesize plastic foams, alkyd resins, and biodiesel. On the other hand, soybean oil is an edible vegetable oil widely used in cooking and food formulations. *D. innoxia* seed neutral oil can be used, after refining, as a good alternative feedstock, edible and non-edible, for the industrial sector of soybean oil.

For DSOI, unusual cyclic fatty acids such as epoxy fatty acids (EFAs), dihydroxy fatty acids (DHFAs), and cyclopropane fatty acids (CPFAs) are identified. The CPFAs are 2-[(2-pentylcyclopropyl)methyl]cyclopropanoic acid (6.9%) and 2-[[2-(2-ethylcyclopropyl)methyl]cyclopropylmethyl]cyclopropanoic acid (6.3%) acid, while the DHFA and EFA 9,10-dihydroxystearic acid (1.22%) and 9,10-epoxystearic acid (ESA, 15.4%), respectively, are also present.

Ahmad reported that DHFAs are formed either from oleic acid by hydroxylation or from EFAs by hydrolysis (EFA is synthesized by the epoxidation of oleic acid). The

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**Fig. 1** Extraction process of *D. innoxia* seeds.
observed difference between the oleic acid contents in DSOH (31.5%) and DSOI (12.8%) may be due to its transformation into EFSA (15.4%). Moreover, the unsaturated fatty acid content of DSOI (20.8%) is much lower than that in DSOH (80.0%). EFSA is used for various industrial processes as plasticizers, stabilizers, diluents, and lubricants [28–30].

Bao et al. [31] reported that CPFAs are synthesized from unsaturated fatty acids by cyclopropane synthase, an enzyme that catalyzes the addition of a methylene group to a double bond in an unsaturated fatty acid. A possible explanation for the presence of CPFAs could be the lack of linoleic and linolenic acids in DSOH (7.60% and 0%) compared to DSOH (46.2% and 0.45%).

As shown in Table 2, the myristic (C14:0), palmitic (C16:0), margaric (C17:0), stearic (C18:0), and arachidic (C20:0) acid contents in DSOI (0.8%, 40.2%, 0.37%, 7.0%, and 0.53%, respectively) are significantly higher (p < 0.05) than those in DSOH (0.38%, 15.2%, 0.12%, 3.2%, and 0.38%, respectively). The iodine values of DSOH, DSOI, and DSOI are 110.0, 110.7, and 24.3 I2/100 g oil. As shown in Table 2, the fatty acid profiles reflect the iodine values. The low iodine value for DSOI reflects the high degree of saturation in the triacylglycerols of the neutral oil (purified oil).

Table 3 shows summaries of the fatty acid profiles of D. innoxia seed oils obtained with hexane (crude oil; DPOH), chloroform (crude oil; DPOC), and isopropanol (purified oil; DPOI) as extracting solvents. As shown in Table 2, DPOH, DPOC, and DPOI are rich in short-chain fatty acids, mainly

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Table 1 The characteristics of D. innoxia seeds and their seed and pod extracts and oils.

| Seed extract | HE | CE | IE |
|--------------|----|----|----|
| Yield (%)    | 11.9 ± 0.6 | 12.3 ± 0.7 | 30.6 ± 1.2 |
| Acidity (%)  | 0.67 ± 0.04 | 0.85 ± 0.05 | 47.1 ± 2.5 |

| Pod extract | HE | CE | IE |
|-------------|----|----|----|
| Yield (%)   | 3.3 ± 0.2 | 3.0 ± 0.2 | 12.7 ± 0.1 |

HE: Hexane extract; CE: Chloroform extract; IE: Isopropanol extract.

DSOH: D. innoxia seed oil extracted with hexane; DSOH: D. innoxia seed oil extracted with chloroform; DSOI: D. innoxia seed oil extracted with isopropanol.

Values having different superscripts in a row are significantly different p < 0.05.
Table 2  Fatty acids profile (wt.%) of crude and purified oils of DSOH, DSOC, and DSOI.

| Fatty acid        | Crude oil DSOH | Crude oil DSOC | Purified oil DSOI |
|-------------------|----------------|----------------|------------------|
|                   | Mean | SD  | Mean | SD  | Mean | SD  |
| C12:0             | 0.16 | 0.01| 0.01 | 0.00| –    | –   |
| C14:0             | 0.38 | 0.02| 0.32 | 0.01| 0.79 | 0.03|
| C16:0             | 15.2 | 0.5 | 14.9 | 0.5 | 40.4 | 1.2 |
| C16:1Δ7c          | –    | 0.03| 0.00| –   | –    | –   |
| C16:1Δ9c          | 0.55 | 0.02| 0.57 | 0.02| –    | –   |
| C16:1Δ11c         | –    | 0.02| 0.00| –   | –    | –   |
| C17:0             | 0.12 | 0.01| 0.11 | 0.01| 0.37 | 0.02|
| C17:1Δ10c         | 0.07 | 0.00| 0.07 | 0.00| –    | –   |
| C18:0             | 3.2  | 0.1 | 3.0  | 0.1 | 7.0  | 0.3 |
| C18:1Δ9c          | 31.5 | 1.0 | 32.4 | 1.0 | 12.8 | 0.4 |
| C18:1Δ11c         | 1.30 | 0.04| 1.4  | 0.1 | 0.43 | 0.02|
| C18:2Δ9t,12t      | 0.03 | 0.00| 0.05 | 0.00| –    | –   |
| C18:2Δ9c,12e      | 46.2 | 1.4 | 46.0 | 1.4 | 7.6  | 0.3 |
| C20:0             | 0.38 | 0.02| 0.37 | 0.02| 0.53 | 0.02|
| C18:3Δ9,12,15     | 0.45 | 0.02| 0.46 | 0.02| –    | –   |
| 9,10-epoxyC18:0   | –    | –   | –    | 15.4| 0.5  |     |
| 3-CyC18:0         | –    | –   | –    | 6.3 | 0.2  |     |
| 2-CyC18:0         | –    | –   | –    | 6.9 | 0.2  |     |
| 9,10-dihydroxyC18:0 | –  | –  | –    | 1.22| 0.04 |     |
| C22:0             | 0.20 | 0.01| 0.19 | 0.01| 0.47 | 0.02|
| C24:0             | 0.18 | 0.01| 0.18 | 0.01| –    | –   |
| C25:0             | 0.03 | 0.00| 0.02 | 0.00| –    | –   |
| C26:0             | 0.04 | 0.00| 0.03 | 0.00| –    | –   |
| MCFA              | 0.5  | 0.3 | 0.8  |     |      |     |
| LCFA              | 99.5 | 99.7| 99.2 |     |      |     |
| UFA               | 80.0 | 81.0| 20.8 |     |      |     |
| MUFA              | 33.4 | 34.5| 13.2 |     |      |     |
| PUFA              | 46.7 | 46.5| 7.6  |     |      |     |
| SFA               | 19.9 | 19.0| 79.2 |     |      |     |
| CFA               | 0    | 0   | 28.6 |     |      |     |

SD: standard deviation; –: not detected; CFA: cyclic fatty acids; MCFA: medium-chain fatty acids; LCFA: long-chain fatty acids; OCFA: odd-chain fatty acid, UFA: unsaturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; 3-CyC18:0: 3-[(2-ethylcyclopropyl)methyl]cyclopropyl]cyclopropaneoctanoic acid; 2-CyC18:0: 2-[(2-pentylcyclopropyl)methyl]cyclopropaneoctanoic acid. Values having different superscripts in a row are significantly different p < 0.05.

caprylic acid (C6:0). The short-chain fatty acid content is significantly higher (p < 0.05) in DPOC (76.2%) than in DPOH (69.1%) and DPOI (70.2%). The caprylic acid content is significantly higher (p < 0.05) in DPOI (48.6%) than in DPOH (38.9%) and DPOC (41.4%). The second most abundant fatty acids are caprylic acid (C8:0), pelar-
gonic acid (C9:0), and oleic acid (C18:1Δ9c) for DPOH (10.17%), DPOC (12.3%), and DPOI (17.7%), respectively. Moreover, the oleic acid content is significantly affected by the type of solvent. The oleic acid content is significantly higher (p < 0.05) in DSOI (17.7%) than in DPOH (5.5%), which is in turn significantly higher (p < 0.05) than that in DPOC (1.9%). DPOH contains fatty acids that are not identified in DPOC and DPOI, such as cerotic (C26:0) and 11-octadecynoic acids. Conversely, DPOH and DPOC contain fatty acids that are not detected in DPOI, such as capric (C10:0), lauric (C12:0), linoleic, linolenic, arachidic, 9,10-epoxystearic, behenic (C22:0), and lignoceric (C24:0) acids. Azimova and Glushenkova\(^3\) reported that short-chain fatty acids were identified in plants of the family Asteraceae, such as the genera *Ajania fastigia* and *Artemisia absinthium* L.

Table 3: Fatty acids profile (wt. %) of crude and purified oils of DPOH, DPOC, and DPOI.

| Fatty acid          | Crude oil DPOH | Crude oil DPOC | Purified oil DPOI |
|---------------------|----------------|----------------|-------------------|
|                     | Mean | SD | Mean | SD | Mean | SD |
| C5:0                | 2.4 \(^b\) | 0.1 | 2.5 \(^b\) | 0.1 | 4.9 \(^a\) | 0.1 |
| C6:0                | 38.9 \(^b\) | 1.2 | 41.4 \(^b\) | 1.3 | 48.6 \(^a\) | 1.5 |
| C7:0                | 8.2 \(^b\) | 0.3 | 8.5 \(^b\) | 0.3 | 7.0 \(^b\) | 0.2 |
| C8:0                | 10.2 \(^b\) | 0.3 | 11.5 \(^a\) | 0.4 | 4.5 \(^a\) | 0.2 |
| C9:0                | 9.4 \(^a\) | 0.3 | 12.3 \(^b\) | 0.4 | 5.2 \(^a\) | 0.2 |
| C10:0               | 1.7 \(^b\) | 0.1 | 2.2 \(^a\) | 0.1 | — | — |
| C12:0               | 0.85 \(^a\) | 0.03 | 0.62 \(^b\) | 0.02 | — | — |
| C14:0               | 0.71 \(^a\) | 0.03 | 0.54 \(^a\) | 0.02 | 3.1 \(^b\) | 0.1 |
| C15:0               | 0.33 \(^b\) | 0.01 | 0.27 \(^b\) | 0.01 | — | — |
| C16:0               | 8.8 \(^b\) | 0.3 | 7.0 \(^b\) | 0.2 | 9.2 \(^b\) | 0.3 |
| C17:0               | 0.54 \(^a\) | 0.02 | 0.63 \(^a\) | 0.02 | — | — |
| C18:0               | 3.4 \(^b\) | 0.1 | 3.0 \(^b\) | 0.1 | — | — |
| C18:1Δ9c            | 5.5 \(^b\) | 0.2 | 1.9 \(^a\) | 0.1 | 17.7 \(^a\) | 0.5 |
| C18:2Δ9c,12c        | 2.9 \(^b\) | 0.1 | 3.0 \(^b\) | 0.1 | — | — |
| C20:0               | 0.99 \(^a\) | 0.03 | 0.86 \(^b\) | 0.03 | — | — |
| C18:3A9,12,15       | 1.7 \(^b\) | 0.1 | 1.5 \(^b\) | 0.1 | — | — |
| 9,10-epoxyC18:0     | 0.51 \(^b\) | 0.02 | 0.24 \(^b\) | 0.01 | — | — |
| C22:0               | 1.40 \(^b\) | 0.04 | 1.00 \(^b\) | 0.03 | — | — |
| C24:0               | 1.20 \(^b\) | 0.04 | 0.63 \(^b\) | 0.02 | — | — |
| C26:0               | 0.15 | 0.01 | — | — | — | — |
| 11-octadecynoic     | 0.17 | 0.01 | — | — | — | — |
| SCFA                | 69.1 | 76.2 | 70.2 | — | — | — |
| MCFA                | 3.6 | 3.7 | 3.1 | — | — | — |
| LCFA                | 27.3 | 19.8 | 26.9 | — | — | — |
| OCFA                | 20.9 | 24.2 | 17.0 | — | — | — |
| UFA                 | 5.5 | 1.9 | 17.7 | — | — | — |
| PUFA                | 4.6 | 4.5 | 0 | — | — | — |
| SFA                 | 89.7 | 93.6 | 82.3 | — | — | — |

SD: standard deviation; –: not detected; SCFA: short-chain fatty acids; MCFA: medium-chain fatty acids; LCFA: long-chain fatty acid; OCFA: odd-chain fatty acid, UFA: unsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

Values having different superscripts in a row are significantly different (p < 0.05).
The yields of the extracts of D. innoxia seeds obtained using hexane, chloroform, and isopropanol are 11.9%, 12.3%, and 30.6%, respectively. The contents of free fatty acids in the extracts are 0.67%, 0.85%, and 47.1% (as oleic), respectively. Thus, the hexane and chloroform extracts are mainly composed of triacylglycerols (crude oils), whereas the isopropanol extract is mainly composed of organic acids. The overall lipid fraction of the isopropanol extract (neutral oil) is 15.8%, which is significantly higher \((p<0.05)\) than those of the hexane- and chloroform-extracted crude oils. The oil content (11.9%) of D. innoxia is significantly higher than that reported by Ramadan et al. \(^\text{14}\) (4.17%) using hexane as an extraction solvent. This finding is somewhat surprising given the fact that other studies have shown that the maximum oil yield is obtained using hexane\(^\text{4, 8}\) or petroleum ether\(^\text{10}\) as the extraction solvent rather than ethyl acetate, chloroform, or ethanol. Bhutada seed oil is rich in both oleic fatty acid value of the isopropanol extract \(\uparrow\) are palmitic acid dicarboxylic acids, alcohols, esters, ketones, aldehydes, and organic acids. The overall lipid fraction of the isopropanol extract is classified into five classes: oxo-, hydroxyl-, epoxy-, simple, and halogeno-fatty acids\(^\text{Table 3}\). Almahd\(^\text{30}\) reported that azelaiac and pelargonic acids are synthesized by oxidative scission of oleic acid. Furthermore, azelaic acid is used in various applications such as plasticizers, lubricants, hydraulic fluids, and polymers as well as being a component of hair and skin conditioners\(^\text{34}\).

The levels of squalene in the unsaponifiable material in DSOH, DSOC, and DSOI are 900, 900, and 520 mg/kg, respectively. No significant differences \((p>0.05)\) are found between the levels of squalene in DSOH and DSOI. However, the level of squalene in DSOI is significantly lower \((p<0.05)\) than those in DSOH and DSOC. Gunstone et al. \(^\text{35}\) reported that the levels of squalene in vegetable oils ranged from 50 to 500 mg/kg and from 700 to 12,000 mg/kg in olive oils.

The results shown in Table 1 indicate that there is no significant difference \((p>0.05)\) between the amounts of carotenoids in DSOH (2.3 mg/kg) and DSOC (2.3 mg/kg). However, the amount of carotenoids in DSOC (1.6 mg/kg) is significantly lower \((p<0.05)\) than those in DSOH and DSOI. In addition, the amount of chlorophylls is significantly higher \((p<0.05)\) in DSOH (4.6 mg/kg) than in DSOC (2.2 mg/kg), which is in turn significantly higher \((p<0.05)\) than that in DSOI (1.4 mg/kg). Oil color is related to the presence of chlorophylls and carotenoids.

In addition to squalene, carotenoids, and chlorophylls, extracted DSOs contain trace amounts of waxes. Two wax compounds are observed in the present study: cetyl palmitate and myristyl stearate. The difference between the amounts of carotenoids in DSOH, DSOC, and DSOI is remarkably significant. These results agree with those reported by Holser\(^\text{36}\), who demonstrated that the solubility of wax compounds in alcohol increases with increasing temperature. The boiling point of isopropanol is 82.5°C. DSOs have a low content of wax compounds compared with other seed oils extracted with hexane, such as sunflower seed oil (1.5–3 wt.%), and rice bean oil (3–4 wt.%).\(^\text{17}\).

### 3.1 Tocopherol contents

Table 5 summarizes the vitamin E contents of the different DSOs. The vitamin E in DSOH, DSOC, and DSOI is found to comprise three isomers, including \(\alpha\)-, \(\gamma\)-, and \(\delta\)-tocopherols. \(\gamma\)-Tocopherol is found to be the predominant isomer (94–98% of the total vitamin E content) in crude and refined oils. These results are in accord with a previous study in which \(\alpha\)-, \(\gamma\)-, and \(\delta\)-tocopherols were detected in the seed oils of the Datura species (including innoxia) and \(\gamma\)-tocopherol was shown to be the major isomer\(^\text{14}\). The total tocopherol content is significantly higher \((p<0.05)\) in DSOH (120 mg/100 g oil) than in DSOC (115 mg/100 g oil) and DSOI (102 mg/100 g oil). The total tocopherol level is lower than that found by Ramadan et al. \(^\text{14}\) in DSO (4.26 g/
kg oil). The total level is higher than those found in other seed oils, such as tea seed oil extracted with hexane (16.88 mg/100 g oil) and isopropanol (23.84 mg/100 g oil). Several studies have demonstrated that the use of alcohols, such as ethanol and isopropanol, in oil extraction yields oils with higher contents of tocopherols than those of oils extracted with hexane. However, several other studies have shown the contrary. This result may be explained by the fact that tocopherols are very nonpolar compounds and are thus fat-soluble vitamins. Shahidi reported that the antioxidant activity of tocopherols is related to the presence of polyunsaturated fatty acids in the seed oil. As shown in Table 2, the total polyunsaturated fatty acid content (linoleic and linolenic) drops from 46.7% (DSOH) to 7.6% (DSO).

Tocopherols are known as biological antioxidants by protecting polyunsaturated fatty acids from peroxidative degeneration. The breakdown products of hydroperoxides are aldehydes and ketones, which produce the disagreeable odors and flavors of rancidity. In food, tocopherols are used as antioxidant for frying oil, margarine, and fried snacks. On the other hand, tocopherols are also classified as a preventive factor for cardiovascular disease, cancer, Alzheimer’s disease, and reduce the risk of degenerative disease.

### Table 4: Identified compounds of the non-hexane fraction of isopropanol extract.

| Composition                        | %    | Composition                        | %    |
|------------------------------------|------|------------------------------------|------|
| Valeric acid (C5:0)                | 0.50 | 8-Oxooctanoic acid                 | 1.0  |
| 1,4-dimethylbenzene                | 0.48 | Azelaic acid                       | 15   |
| Allyl ethyl carbonate              | 0.19 | Stearic acid                       | 5.3  |
| n-butyl ethanoate                  | 0.16 | 9-hydroxyacapric acid              | 4.6  |
| 1,3-dimethylbenzene                | 0.61 | Oleic acid                         | 4.0  |
| Caproaldehyde                      | 0.68 | Sebacic acid                       | 0.29 |
| Capric acid (C6:0)                 | 5.2  | Linoleic acid                      | 3.7  |
| 1,2,3-trimethylbenzene             | 0.17 | Eicosanoic acid                    | 0.46 |
| heptanal                           | 0.20 | Undecanedioic acid                 | 0.46 |
| 2-heptanone                        | 0.08 | Docosanoic                         | 0.20 |
| Enanthic acid (C7:0)               | 0.52 | 9,10-dibromoocatadacanoic acid     | 2.4  |
| 1,3,5-trimethylbenzene             | 0.22 | 4-Oxodecanolic acid                | 0.35 |
| Octanal                            | 0.42 | Tetracosanoic                      | 0.21 |
| Caprylic acid (C8:0)               | 1.2  | 9,10-epoxystearic acid             | 0.28 |
| Nonanal                            | 6.0  | 9,10-dihydroxystearic acid         | 1.6  |
| Pelargonic acid (C9:0)             | 6.0  | 10-Oxoocadecanoic acid             | 0.45 |
| β-Hydroxy-isovaleric acid          | 0.05 | 4-oxo-azelaic acid                 | 0.13 |
| 1-octanol                          | 0.25 | Function group                     |      |
| Malonic acid                       | 0.38 |                                  |      |
| 2-Methyl propyl 2-methyl 2-butenoate | 0.15 | Aromatic                           | 1.5  |
| Succinic acid                      | 0.47 | Aldehyde                           | 7.7  |
| Lauric acid                        | 0.37 | Ester                              | 0.50 |
| Myristic acid                      | 0.84 | Ketone                             | 0.08 |
| Stearaldehyde                      | 0.36 | Fatty alcohol                      | 0.25 |
| Pentadecanoic acid                 | 0.12 | Diacid                             | 18   |
| Methoxyacetic acid                 | 0.10 | Fatty acid                         | 72   |
| Palmitic acid                      | 0.10 | Oxo-fatty acid                     | 1.8  |
| Palmitic acid                      | 0.10 | Hydroxy-fatty acid                 | 6.2  |
| Suberic acid                       | 1.7  | Epoxy-fatty acid                   | 0.28 |
| margaric acid                      | 0.18 | Simple-fatty acid                  | 60   |
|                                 |      | Halogeno-fatty acid                | 3.4  |

%: in weight percent.
arising from oxidative stress.\(^{30}\)

### 3.2 Sterol contents

Table 5 compares the results obtained for sterol analysis of the unsaponifiable fractions of DSOH, DSOC, and DSOI. The total sterol content is significantly higher (\(p < 0.05\)) in DSOI (14,000 mg/kg oil) than in DSOH (11,500 mg/kg oil) and DSOC (12,600 mg/kg oil). No significant difference (\(p > 0.05\)) is found between the total sterol contents of DSOH and DSOC. In accordance with the present results, previous studies have demonstrated that the total sterol content obtained using an alcohol as the extraction solvent is higher than that achieved using hexane.\(^{30}\) Cheng et al.\(^{40}\) reported that the high polarity of alcohols promotes the extraction of hydrophilic compounds such as sterols compared to that achieved with nonpolar solvents. The major sterol components identified are stigmasterol (29%–31%), \(\beta\)-sitosterol (21%–22%), 24-nor-22,23-methylenecholesterol-5-en-3\(\beta\)-ol (15%), cycloartenol (6.2%–6.5%), lanosterol (5.5%–6.0%), \(\Delta^\prime\)-avenosterol (5.6%–5.7%), gramisterol (4.9%–5.3%), and campesterol (4.4%–5.8%). The amount of total sterol is lower than that in Egyptian DSO (16.76 g/kg oil)\(^{14}\). However, the total sterol is higher than those of other seed oils, such as castor seed oil (\(\approx 2,500\) mg/kg oil)\(^{30}\). Among the 14 sterol isomers identified in the different oils in the present study (Table 5), only five were identified in Egyptian DSO, those being campesterol, stigmasterol, lanosterol, \(\beta\)-sitosterol, and \(\Delta^\prime\)-Avenosterol. Similarly, sigmasterol and \(\beta\)-sitosterol are the predominant components found in both oils, accounting for approximately 50% (Saudi DSO) and 70% (Egyptian DSO).

Sterols are isopropanoid lipids essential to cell membrane structure and function, and to a further metabolism into steroidal hormones in eukaryotes. Moreover, Mckeen et al.\(^{26}\) reported that foods containing sterols reduce the risk of coronary heart disease. In addition, a daily dietary intake of phytosterols of 800 mg or more is necessary to reduce the risk of coronary heart disease, the phytosterol

### Table 5

Sterol and tocopherol compositions of crude and purified oils of DSOH, DSOC, and DSOI.

| Sterols | Crude oil DSOH | Crude oil DSOC | Purified oil DSOI |
|---------|----------------|----------------|------------------|
|         | Mean          | SD  | %  | Mean | SD  | %  | Mean | SD  | %  |
| Stigmasterol | 3600\(^b\) | 120 | 31 | 3800\(^ab\) | 120 | 31 | 4100\(^a\) | 130 | 29 |
| \(\beta\)-Sitosterol | 2500\(^b\) | 81 | 22 | 2800\(^a\) | 89 | 22 | 3000\(^a\) | 96 | 21 |
| 24-Nor-22,23-methylenecholesterol-5-en-3\(\beta\)-ol | 1700\(^b\) | 54 | 15 | 1900\(^b\) | 61 | 15 | 2100\(^a\) | 68 | 15 |
| Cycloartenol | 710\(^b\) | 23 | 6.2 | 810\(^a\) | 26 | 6.5 | 870\(^a\) | 28 | 6.2 |
| Lanosterol | 700\(^b\) | 22 | 6.1 | 720\(^a\) | 23 | 5.7 | 770\(^a\) | 24 | 5.5 |
| \(\Delta^\prime\)-Avenosterol | 640\(^b\) | 21 | 5.6 | 720\(^b\) | 23 | 5.7 | 790\(^a\) | 25 | 5.7 |
| Gramisterol | 590\(^b\) | 19 | 5.1 | 670\(^a\) | 21 | 5.3 | 686\(^a\) | 22 | 4.9 |
| Campesterol | 500\(^b\) | 16 | 4.4 | 570\(^b\) | 18 | 4.6 | 810\(^a\) | 26 | 5.8 |
| Desmosterol | 170\(^b\) | 6.0 | 1.5 | 180\(^b\) | 6.0 | 1.5 | 260\(^a\) | 8.0 | 1.9 |
| 7\(\alpha\),8-epoxy lanost-9(11)-en-3\(\beta\)-ol acetate | 140\(^b\) | 4.5 | 1.2 | 140\(^b\) | 5.0 | 1.1 | 160\(^a\) | 5.0 | 1.1 |
| 3-acetoxy-24-norchol-5-ene | 120\(^b\) | 4.0 | 1.1 | 120\(^b\) | 3.0 | 1.0 | 280\(^a\) | 9.0 | 2.0 |
| 7\(\beta\)-hydroxycholesterol | 45\(^b\) | 1.5 | 0.4 | 41\(^b\) | 1.3 | 0.3 | 71\(^a\) | 2.2 | 0.5 |
| Gorgosterol | 36\(^b\) | 1.1 | 0.3 | 47\(^a\) | 1.5 | 0.4 | 21\(^a\) | 0.7 | 0.2 |
| 4\(\beta\)-hydroxycholesterol | 16\(^b\) | 0.5 | 0.1 | 17\(^b\) | 0.6 | 0.1 | 19\(^a\) | 0.6 | 0.1 |
| Total (mg/kg) | 11500\(^b\) | 370 | 100 | 12600\(^b\) | 420 | 100 | 14000\(^a\) | 450 | 100 |

Tocopherols

|         | Crude oil DSOH | Crude oil DSOC | Purified oil DSOI |
|---------|----------------|----------------|------------------|
|         | Mean          | SD  | %  | Mean | SD  | %  | Mean | SD  | %  |
| \(\alpha\)-T | – | – | 2.8 | 0.1 | 2.8 |
| \(\gamma\)-T | 120\(^a\) | 4.3 | 98 | 110\(^b\) | 3.8 | 94 | 95\(^a\) | 3.4 | 94 |
| \(\delta\)-T | 2.7\(^a\) | 0.1 | 2.2 | 7\(^a\) | 0.3 | 6.1 | 3.6\(^b\) | 0.1 | 3.6 |
| Total (g/100 g) | 120 | 4.5 | 100 | 115 | 4.0 | 100 | 102 | 3.6 | 100 |

SD: standard deviation; -: not detected; Values having different superscripts in a row are significantly different \(p < 0.05\) %: in weight percent.

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mixtures should mainly contain high level of β-sitosterol, campesterol, and stigmasterol. *Datura innoxia* seed oils contain high level of sterols and β-sitosterol, campesterol, and stigmasterol are present with a high percentage.

### 4 Conclusions

These findings reveal the effect of solvent type on the composition of crude and purified DSOs extracted with hexane, chloroform, and isopropanol. The results of this study show that neutral oil of purified DSO extracted with isopropanol contains a significant amount of unusual fatty acids and free fatty acids. The unusual fatty acids are epoxo, dihydroxy, and cyclopropane fatty acids, which are not detected in the crude oils extracted with hexane and chloroform. Moreover, *D. innoxia* seeds yield a crude and refined oils rich in tocopherols and sterols, with the tocopherol content being high in the crude oil hexane extract and the sterols content being high in the purified oil of isopropanol extract. For edible purpose, using hexane as the extraction solvent is the good process to obtain valuable fatty acids such oleic and linoleic acids. Moreover, these fatty acids can be used for polymer industry after epoxidation reaction. Using isopropanol as the extraction solvent is good for biodiesel production. No significant difference between chloroform and hexane extract in fatty acid composition. As a result, chloroform is not likely to be used as replacement for hexane for edible oil extraction due to its degree of toxicity.

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### Conflict of Interest

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

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