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Screening of bioactive lipids and radical scavenging potential of some solanaceae plants

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Abstract

Gas-liquid chromatography (GLC) and normal-phase high performance liquid chromatography (HPLC) were used to analyse fatty acids and fat-soluble bioactives of the seeds of four *Datura* species (*D. stramonium*, *D. metel*, *D. tatula*, *D. innoxia*) and two *Hyoscyamus* species (*H. muticus*, *H. niger*). The amounts of *n*-hexane extract were found to be between 5.50% and 12.6%. The major fatty acid was linoleic acid followed by oleic, palmitic and stearic acids. The crude *n*-hexane extract was characterised by a relatively high amount of phytosterols, wherein the sterol markers were stigmasterol, β-sitosterol, lanosterol, Δ5-avenasterol and sitostanol. In all plant extracts γ-tocopherol was the major component present accounting for more than 80% of total tocopherols detected. When different extracts were compared for their radical scavenging activity (RSA) toward the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, *D. Innoxia* exhibited a stronger RSA.

Keywords: Chromatographic analysis; Fatty acids; Lipid-soluble bioactives; Radical scavenging activity; DPPH; Solanaceae

1. Introduction

Datura and *Hyoscaymus* plants have been a focus of numerous biological investigations both *in vivo* and *in vitro* due to their wide medicinal use. As they contain important anticholinergic tropane alkaloids (Adrian et al., 1990; Berkov & Zayed, 2004; Iliana et al., 1994; Rosa et al., 2000; Zayed, 2001, 2003; Zayed, Abbas, & Wink, 2003; Zayed & Wink, 2004). In the present work, attempts were made to study and analyze fatty acids and fat-soluble bioactives of the seeds of these plants.

A large quantity of bioactive oils and fats whether for human consumption or for industrial purposes is presently derived from plant sources. To meet the increasing demand for oils, improvements are being made with conventional crops as well as with selected plant species that have the ability to produce unique desirable oils. Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance. Several plants are now grown not only for food and fodder but also for a variety of products with application in industry, including oils and pharmaceuticals. Natural fats and oils contain, apart from acylglycerols, a number of lipophilic materials with a very diverse chemical make up. Among the most interesting ones are the sterols and fat-soluble vitamins. In this work, fatty acids and fat-soluble bioactives of *Datura* and *Hyoscyamus* have been analysed.

On the other side, plants rich in antioxidants provide protection against cancer and cardio- and cerebrovascular diseases through their capacity to scavenge free radicals. Moreover, lipid oxidation occurs through free radical chain process *via* initiation, propagation and termination steps. Antioxidants may function as free radical scavengers and as quenchers of the formation of singlet oxygen. It is hard

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to find data on the radical scavenging activity (RSA) and antioxidant potential of plants under study. Recently, Ramadan, Kroh, and Moersel (2003) developed a fast method for testing the antiradical performance of lipids.

The objective of this investigation was to obtain information about the chemical nature of lipid fraction of the seeds of plants under study, which may serve as a basis for further detailed chemical investigation and nutritional evaluation. Moreover, antiradical action of plants extract was assayed to screen their antioxidant potential. The results will be important as an indication of the potentially nutraceutical and economical utility of these plant seeds.

2. Material and methods

2.1. Materials

Seeds of *Datura* and *Hyoscyamus* were collected in February 2004 from the experimental garden, Faculty of Pharmacy and Faculty of Agriculture, Zagazig University, Egypt. Standards used for sterols (ST) characterisation were purchased from Supelco (Bellefonte, PA, USA). Standards used for vitamin E (α-, β-, γ- and δ-tocopherol) were purchased from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH, approximately 90%) was from Sigma (St. Louis, MO, USA). Reagents and chemicals used were of the highest purity available.

2.2. Methods

2.2.1. Solvent extraction of total lipids (TL)

Plant material were finely ground (particle size = 0.2 mm) and Soxhlet extracted with *n*-hexane for 8 h. Total lipids (TL) recovered were weighed and stored at 4 °C for further analysis.

2.2.2. Gas chromatography (GC) analysis of fatty acid methyl esters

Fatty acids were transesterified into methyl esters (FAME) using *N*-trimethylsulphonium hydroxide (Macherey-Nagel, Düren, Germany) according to the procedure reported by Arens, Schulte, and Weber (1994). FAME were identified using a Shimadzu GC-14A equipped with flame ionisation detector (FID) and C-R4AX chromatopac integrator (Kyoto, Japan). The flow rate of the carrier gas helium was 0.6 mL/min and the split value with a ratio of 1:40. A sample of 1 μL was injected on a 30 m × 0.25 mm × 0.2 μm film thickness Supelco SP™-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature were set at 250 °C. The initial column temperature was 100 °C programmed at 5 °C/min until 175 °C and kept 10 min at 175 °C, then 8 °C/min until 220 °C and kept 10 min at 220 °C. A comparison between the retention times of the samples with those of authentic standard mixture (Sigma, St. Louis, MO, USA; 99% purity specific for GLC), run on the same column under the same conditions, was made to facilitate identification.

2.2.3. Gas chromatography (GC) analysis of sterols (ST)

Separation of ST was performed after saponification of the oil sample without derivatisation according to Ramadan and Moersel (2003). Total lipids (250 mg) were refluxed with 5 mL ethanolic potassium hydroxide solution (6%, w/v) and a few anti-bumping granules for 60 min. The unsaponifiables were first extracted three times with 10 mL of petroleum ether (40–60°, Carl Roth GmbH, Karlsruhe, Germany), the extracts were combined and washed three times with 10 mL of neutral ethanol/water (1:1, v/v) and then dried overnight over anhydrous sodium sulphate. The extract was evaporated using a rotary evaporator at 25 °C under reduced pressure and then ether was completely evaporated under nitrogen. GLC analyses of unsaponifiable residues were carried out using a Mega Series (HRGC 5160, Carlo Erba Strumentazione; Milan, Italy) equipped with FID. The following parameters were used: DB 5 column (J& W scientific; Falsom, CA, USA) packed with 5% phenylmethylpolysiloxan, 30 m length, 0.25 mm i.d., 1.0 μm film thickness; carrier gas (helium) flow 38 mL/min (split–splitless injection was used). Detector and injector were set at 280 °C. The oven temperature was kept constant at 310 °C and the injected volume was 2 μL. The repeatability of the analytical procedure was tested and the relative standard deviation of three repeated analyses of a single sample was <5%. Quantitative analyses were performed with a Shimadzu (C-R6A Chromatopac; Kyoto, Japan) integrator.

2.2.4. Normal phase high performance liquid chromatography (NP-HPLC) separation, identification and quantification of tocopherols

2.2.4.1. Procedure. NP-HPLC was selected to avoid extra sample treatment (e.g., saponification) according to Ramadan and Moersel (2002). Analysis was performed with a solvent delivery LC-9A HPLC (Shimadzu, Kyoto, Japan). The chromatographic system included a model 87.00 variable wavelength detector and a 250 × 4 mm i.d. LiChrospher-Si 60, 5 μm, column (Knauer, Berlin, Germany). Separation of tocopherol homologues was based on isocratic elution when the solvent flow rate was maintained at 1 mL/min at a column back-pressure of about 65–70 bar. The solvent system selected for elution was isooctane/ethyl acetate (96:4, v/v) with detection at 295 nm. Twenty microlitres of the diluted solution of TL in the mobile phase was directly injected into the HPLC column. Tocopherol homologues were identified by comparing their retention times with those of authentic standards.

2.2.4.2. Preparation of standard curves. Standard solutions were prepared by serial dilution to concentration of approximately 5 mg $mL^{-1}$of each tocopherol homologue. Standard solutions were prepared from a stock solution which was stored in the dark at −20 °C. Twenty microlitres was injected and peaks areas were determined to generate standard curve data.

2.2.4.3. Quantification. All quantitation was by peak area using a Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Standard curves were calculated from six concentrations levels by linear regression. Based on the established chromatographic conditions, repeated injections of different concentrations of the standard tocopherols were made three times onto the HPLC system. Injections in triplicate were made at each concentration for both standards and samples. All work was carried out under subdued light conditions. All the experiments were repeated at least thrice when the variation on any one was routinely less than 5%.

2.2.5. Radical scavenging activity (RSA) of plants extracts toward DPPH radical

Different solvents were used to assay the RSA of plant lipophilic extracts, whereas the best results was obtained with toluene which was able to dissolve completely the hydrophobic and the hydrophilic compounds (Ramadan et al., 2003; Ramadan-Hassanien, 2004). Therefore, the RSA of different plant oils was assayed with DPPH radical previously dissolved in toluene. Toluenic solution of DPPH radicals was freshly prepared at a concentration of 10^{-4} M. The radical, in the absence of antioxidant compounds, was stable for more than 2 h of normal kinetic assay. For evaluation, 10 mg of oil (in 100 μL toluene) was mixed with 390 μL toluenic solution of DPPH radicals and the mixture was vortexed for 20 s at ambient temperature. Against a blank of pure toluene without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 30 and 60 min of mixing using UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without sample (control) and the inhibition percent was calculated according to the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample})/\text{absorbance of control}] \times 100.$$

All experimental procedures were performed in triplicate and their mean values (±standard deviation) are given.

3. Results and discussion

In the present investigation, the amounts of hexane extract were found to be between 5.50% and 12.6% (Table 1). Level of total lipids (TL) was the highest in *H. niger*, while *D. innoxia* recorded the lowest TL amount.

3.1. Fatty acid profile of total lipids (TL)

According to the results shown in Table 1, 13 fatty acids were identified in different extracts, wherein the analysis of FAME gave the proportion of linoleic followed by oleic, palmitic and stearic as the major fatty acids, comprising together more than 95% of total identified FAME. In different plant extracts linoleic acid (C18:2$n-6$) was the dominant oil fatty acid accounting for about 50% or more of the total fatty acid content. The activity of these includes lowering of blood pressure and constriction of smooth muscle. A striking feature of the different extracts was the relatively high level of polyunsaturated fatty acids (PUFA). Trienes [(γ-linolenic acid GLA, C18:3$n-6$) and (α-linolenic acid ALA, C18:3$n-3$)] were also estimated in relatively lower amounts. *Hyoscyamus species* characterised by higher unsaturation ratio while *Datura* characterised by their relatively higher levels of saturated fatty acids. From the fatty acid profile of different plant it could be said that the lipids as a good source of essential fatty acids. Dramatically in recent years, a growing body of literature illustrates the benefits of PUFA in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune

Table 1
Levels of total lipids (g/kg) and their fatty acid profile (%) of different lipids under study

	Datura metel	*D. innoxia*	*D. stramonium*	*D. tatula*	*Hyoscyamus muticus*	*H. niger*
Total lipids (g/kg)	55.0	41.7	103	90	83.7	126
Fatty acid	*Relative content (%)*					
C14:0	0.19 ± 0.02	0.20 ± 0.01	0.15 ± 0.03	0.15 ± 0.03	nd[a]	nd
C16:0	13.8 ± 0.13	14.7 ± 0.15	12.7 ± 0.12	13.3 ± 0.17	9.04 ± 0.10	6.68 ± 0.11
C16:1$n-7$	0.40 ± 0.01	0.35 ± 0.01	0.37 ± 0.02	0.33 ± 0.02	0.44 ± 0.03	0.33 ± 0.04
C18:0	2.00 ± 0.08	2.34 ± 0.09	2.79 ± 0.03	3.18 ± 0.05	2.77 ± 0.09	3.26 ± 0.05
C18:1$n-9$	30.1 ± 0.55	28.4 ± 0.47	26.1 ± 0.50	27.8 ± 0.66	21.3 ± 0.74	15.3 ± 0.67
C18:2$n-6$	51.5 ± 1.02	52.8 ± 0.98	56.4 ± 0.99	54.3 ± 1.22	65.6 ± 1.04	73.2 ± 1.32
C20:0	0.33 ± 0.05	0.37 ± 0.04	0.38 ± 0.04	0.35 ± 0.06	0.37 ± 0.05	0.42 ± 0.03
C18:3$n-3$	0.11 ± 0.02	0.11 ± 0.01	0.10 ± 0.03	0.10 ± 0.02	nd	nd
C18:3$n-6$	nd	nd	nd	nd	0.14 ± 0.02	0.18 ± 0.02
C20:2	0.17 ± 0.03	0.15 ± 0.03	0.15 ± 0.04	0.15 ± 0.04	0.20 ± 0.03	0.23 ± 0.03
C24:0	0.25 ± 0.05	0.25 ± 0.03	0.36 ± 0.03	0.21 ± 0.01	nd	0.20 ± 0.03
Unknown	1.05 ± 0.06	0.33 ± 0.06	0.50 ± 0.04	0.13 ± 0.03	0.14 ± 0.03	0.19 ± 0.05

Results are given as means ± SD from triplicate estimations.
[a] Not detected.

disorder and diabetes (Finley & Shahidi, 2001; Kamel & Kakuda, 2000; Riemersma, 2001). The fatty acid composition and high amounts of PUFA make these lipids a special component for nutraceutical applications.

3.2. Sterol analysis and composition

The analysis of free sterols (ST) provides important information about the quality and the identity of lipids investigated (Artho, Grob, & Marianai, 1993; De-Blas & Del-Valle, 1996; Grob, Laufranchi, & Mariani, 1990; Homberg, 1991; Horstmann & Montag, 1987). In plants, neither cultivation of new breeding lines nor environmental factors alter content and composition of free sterols significantly, in contrast to the fatty acid composition, which has been changed dramatically by breeding programmes (Hirsinger, 1989; Homberg, 1991). A relatively high amount of unsaponifiable (between 2.05% and 4.26% of TL) was recovered from plants extracts. The highest amount of unsaponifiable was found in *D. innoxia* plant extract (42.6 g/kg TL) while the lowest level was recovered from *D. stramonium* (20.5 g/kg TL). Total phytosterols content was recorded at a higher level in *D. innoxia* (1.67% of TL) followed by *D. metel* (1.34% of TL) while the lowest level of total sterols was recovered from *D. stramonium* (0.81% of TL). Nine compounds were identified (Table 2), wherein the sterol marker was stigmasterol in almost all extracts (*ca.* 28.5–52.5% of total sterols). The exception was in *D. tatula*, wherein lanosterol was the major phytosterol (39.0% of total sterols). Generally in all extracts, the next major components were β-sitosterol, lanosterol, Δ5-avenasterol and sitostanol. Other components, e.g., campesterol, Δ7-stigmastenol, Δ7-avenasterol and Δ5, 24-stigmastadinol were present in lower amounts or traces. Among different plant sterols, sitosterol has been most intensively investigated with respect to its beneficial physiological effects in man (Yang, Karlsson, Oksman, & Kallio, 2001). Phytosterols, in general, are of interest due to their antioxidant activity and impact on health. Recently, phytosterols have been added to margarine and vegetable oils as examples of successful functional foods (Ntanios, 2001).

3.3. Tocopherols profile

Tocopherols are the major lipid-soluble, membrane-localised antioxidants in humans. Deficiency of these compounds affects many tissues in mammalian and bird models (Nelson, 1980). Vitamin E deficiency in man causes defects in the developing nervous system in children and haemolysis in man (Sokol, 1996). Eipdemiologic studies suggest that people with lower vitamin E and other antioxidant intake and plasma levels may be at increased risk for certain types of cancer and for atherosclerosis (Gey, Puska, Jordan, & Moser, 1991; Rimm et al., 1993). It is also suggested that supplementation with antioxidants may decrease the risk of these and other degenerative processes (Kallio, Yang, Peippo, Tahvonen, & Pan, 2002). Tocopherols in vegetable oils, moreover, are believed to protect PUFA from peroxidation (Kamal-Eldin & Andersson, 1997). The effectiveness of tocopherols as lipid antioxidants has been attributed mainly to their ability to break chain reactions by reacting with fatty acid peroxyl radicals. Vitamin E provides protection against environmental pollutants such as NO_2 and O_3. Both have been shown to initiate lipid peroxidation while NO_2-initiated autoxidation of unsaturated fatty acids is inhibited by phenolic antioxidants such as α-tocopherol; O_3-initiated autoxidation is only partially inhibited (Ramadan & Moersel, 2002). Data about qualitative and quantitative composition of vitamins E are summarized in Table 3. In this investigation, NP-HPLC technique was used to eliminate column contamination problems and allow the use of a general lipid extract for tocopherols isolation. Thus, saponification of oil sample was not required, which allowed shorter analysis time and greater vitamin stability during analysis. The highest level of total tocopherols was found in *D. innoxia* (0.42% of TL), while the lowest level was recovered from *D. stramonium* (0.20% of TL). In all plant extracts γ-tocopherol was the major tocopherol homologue which accounted for more than 80% of total tocopherols detected. α-Tocopherol is the most efficient antioxidant of tocopherol homologues, while β-tocopherol has 25–50% of the antioxidative activity of α-tocopherol, and γ-isomer 10–35% (Kallio et al., 2002). Despite general agreement that

Table 2
Levels of phytosterols (g/kg oil) in different plant extracts

Compound	*Datura metel*	*D. innoxia*	*D. stramonium*	*D. tatula*	*Hyoscyamus muticus*	*H. niger*
Campesterol	0.083 ± 0.005	0.122 ± 0.050	0.054 ± 0.011	0.050 ± 0.010	0.089 ± 0.010	0.052 ± 0.033
Stigmasterol	7.087 ± 0.33	8.296 ± 0.45	3.362 ± 0.29	2.439 ± 0.22	4.060 ± 0.44	4.212 ± 0.36
Lanosterol	1.058 ± 0.12	1.717 ± 0.15	0.563 ± 0.08	3.338 ± 0.22	1.589 ± 0.12	0.801 ± 0.09
β-Sitosterol	2.025 ± 0.17	3.485 ± 0.22	1.722 ± 0.15	1.643 ± 0.11	2.934 ± 0.19	1.747 ± 0.25
Δ5-Avenasterol	0.390 ± 0.08	0.474 ± 0.05	1.164 ± 0.16	0.479 ± 0.07	0.510 ± 0.05	0.655 ± 0.06
Sitostanol	2.254 ± 0.14	2.244 ± 0.16	0.934 ± 0.12	0.385 ± 0.09	1.554 ± 0.19	1.165 ± 0.11
Δ5, 24 stigmastadienol	nd[a]	nd	nd	nd	0.076 ± 0.05	1.684 ± 0.19
Δ7-stigmastenol	0.089 ± 0.03	0.204 ± 0 .09	nd	nd	nd	nd
Δ7-Avenasterol	0.490 ± 0.04	0.219 ± 0.08	0.358 ± 0.06	0.208 ± 0.05	0.758 ± 0.05	0.073 ± 0.07
Total ST content	13.47	16.76	8.157	8.542	11.57	10.38

Results are given as the average of triplicate determinations ± SD.
[a] Not detected.

Table 3
Levels of tocopherols (g/kg oil) in different plant extracts

Compound	*Datura metel*	*D. innoxia*	*D. stramonium*	*D. tatula*	*Hyoscyamus muticus*	*H. niger*
α-Tocopherol	0.067 ± 0.02	0.127 ± 0.04	0.061 ± 0.02	nd	0.379 ± 0.06	0.261 ± 0.03
β-Tocopherol	nd[a]	nd	nd	nd	0.058 ± 0.05	0.104 ± 0.03
γ-Tocopherol	3.268 ± 0.43	4.047 ± 0.39	1.947 ± 0.25	2.140 ± 0.22	2.379 ± 0.36	2.088 ± 0.40
δ-Tocopherol	0.033 ± 0.01	0.085 ± 0.03	0.041 ± 0.01	nd	0.087 ± 0.02	0.156 ± 0.06
Total tocopherols	3.368	4.259	2.049	2.140	2.903	2.609

Results are given as the average of triplicate determinations ± SD.
[a] Not detected.

α-tocopherol is the most efficient antioxidant and vitamin E homologue *in vivo*, however, studies indicate a considerable discrepancy in its absolute and relative antioxidant effectiveness *in vitro*, especially when compared to γ-tocopherol (Kamal-Eldin & Appelqvist, 1996).

3.4. Radical scavenging activity (RSA) of plants oils

Interest has increased in the past few years in the free radical theory of disease causation, particularly in vascular diseases and certain forms of cancer. These developments have led to the investigation on the antioxidant nutrients in a possible prophylactic, even curative, role in the disease process. Closely related to this probable benefit of natural antioxidants is their role in controlling free radicals as they may lead to pathological effects such as vascular diseases and cancer. Oxidation is a natural and needed reaction in metabolism resulting in highly reactive hydroxyl radicals, OH. These can attack DNA, protein and polyunsaturated fatty acids residues of membrane phospholipids. With the latter, a peroxyl radical is formed. Antioxidants quench this radical. If the supply of antioxidants is inadequate, a chain reaction takes place that may lead to damaged tissue. The evidence in the literature begins to make an overwhelming case for the existence of a relationship between high blood levels of antioxidant and a lowered incidence of disease.

The tests expressing antioxidant potency can be categorised into two groups: assays for radical scavenging ability and assays that test the ability to inhibit lipid oxidation under accelerated conditions. However, the model of scavenging stable free radicals is widely used to evaluate the antioxidant properties in a relatively short time, as compared to other methods (Ramadan et al., 2003; Ramadan & Moersel, 2006). The authors have performed a simple experiment, using the same solvent (toluene) to dissolve the fat or oil samples and the free radicals. This allowed to characterise and compare the RSA of all samples under the same conditions.

Apart from the oxidative stability of oils and fats that depends on the fatty acid composition, the presence of minor fat-soluble bioactives and the initial amount of hydroperoxides are also important. Antiradical properties of the differet oils under study were compared using stable DPPH radical. Fig. 1 shows that *D. innoxia* had the strongest RSA. After 1 h incubation, 60% of DPPH radical was

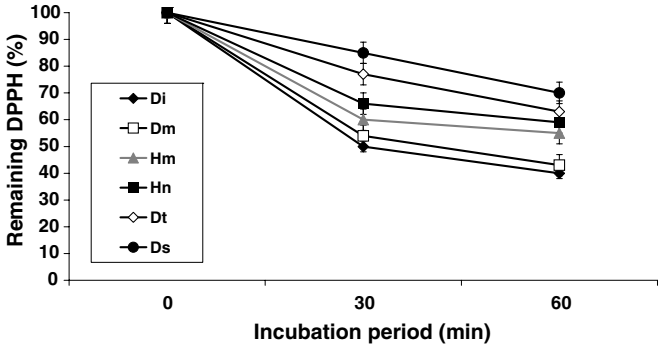

Fig. 1. Scavenging effect at different incubation times of plants oils on DPPH radical as measured by changes in absorbance values at 515 nm. Error bars show the variations of three determinations in terms of standard deviation. Abbreviations; Di, *D. Stramonium*; Dm, *D. metel*; Dt, *D. Tatula*; Di, *D. Innoxia*; Hm, *H. Muticus*; Hn, *H. Niger*.

quenched by *D. innoxia*, while *D. stramonium* was able to quench only 30%. Regarding the composition of different oils, they have different pattern of fatty acid and lipid-soluble bioactives. It could be said that the RSA of oils and fats can be interpreted as the combined action of different endogenous antioxidants. However, when unsaponifiables and polar fractions containing high levels of polar lipids and low levles of phenolics, strong RSA of these components can be expected as well as synergistic activity with primary antioxidants (Ramadan & Moersel, 2006). The stronger antiradical action of *D. innoxia* compared to other oils may be due to (i) the differences in content and composition of polar lipids and unsaponifiable; (ii) the diversity in structural characteristics of potential phenolic antioxidants present; (iii) a synergism of polar lipids with other components present; and (iv) different kinetic behaviours of potential antioxidants. All these factors may contribute to the radical quenching efficiency of oils and fats.

4. Conclusions

Improved knowledge on the composition, analysis and properties of lipid fraction of the seeds related to the plant under study would assist in efforts for nutritional application of these plants. The data about this investigation are very few; on the other hand, there are no reports in the literature about detailed composition of the seeds examined. It could be said that the *Datura* and *Hyoscyamus* seeds give

considerable yield of oil and the oil seems to be a good source of essential fatty acids and lipid-soluble bioactives. Tocopherols and sterols at the level estimated may be of medicinal importance. The preliminary finding of a higher antiradical action of *D. innoxia*, in comparison with other oils, indicates that *D. innoxia* is a potent source of antioxidant compounds which will reflect on its pharmaceutical value as a new unconventional source for pharmaceutical industries and for edible purposes.

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