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Isolation of antioxidant phytoconstituents from the seeds of *Lens culinaris* Medik

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ABSTRACT

Lens culinaris Medik (Leguminosae) is an annual, bushy and herbaceous plant cultivated globally for its edible seeds. A methanolic extract of the seeds contained four new antioxidant compounds, namely β-sitosteryl-3-(2′-*n*-eicosanyloxy)-benzoate (**3**), *n*-octadec-9-enoyl-1-β-D-glucurano-pyranoside (**4**) α-D-galactopyranosyl-(6→1′)-α-D-galactopyranosyl-(6′→1″)-α-D-galactopyranosyl-(6″→1‴)-α-D-galacto-pyranoside (**5**) and benzoyl-*O*-α-D-glucopyranosyl-(2a→1b)-*O*-α-D-glucopyranosyl-(2b→1c)-*O*-α-D-glu-copyranosyl-(6c→1d)-*O*-α-D-glucopyranosyl-(6d→1e)-*O*-α-D-gluco-pyranoside (**6**) along with two known compounds *n*-heptadecanyl *n*-octadec-9-enoate (**1**) and β-sitosterol (**2**) on the basis of chromato-graphic and spectral data analytical techniques. Compound **3** showed significant antioxidant activity compared to compounds **4**, **5**, and **6**.

1. Introduction

Lentil (*Lens culinaris* Medik, Leguminosae) is an ancient crop of classical Mediterranean civilization and continues to play an important role in human health (Zohary & Hopf, 2000). It is an annual, bushy, herbaceous plant mainly grown for its edible seeds (Ford, Rubeena, Redden, Materne, & Taylor, 2007) and commonly known as Adas, Masur, Mercimek and Heramame (Summerfield & Muehlbauer, 1982). It is primarily cultivated in south-eastern Asia for making food items and its flours are used to make culinary dishes in the Asian subcontinent, Middle East, Europe and North America (Williams & Singh, 1988). Western countries use it in casseroles and as a meat substituent in vegetarian diets. It is called as a 'poor man's meat' and is equally liked by all socioeconomic groups (Bhatty, 1988), and has an excellent source of proteins, carbohydrates, fibers, vitamins and minerals (Chibbar, Ambigaipalan, & Hoover, 2010). Potential bioactive compounds such as phytosterols, squalene and tocopherols (Benveniste, 1986; Ryan, Galvin, Connor, Maguire, & Brien, 2007), saponins, flavonoids and tannins (South & Miller, 1998), phytic acid, antinutritional compounds (Zhou & Erdman, 1995) and oligosaccharides (Roberfroid, 2002; Salminen et al., 1998; Swennen, Courtin, & Delcour, 2006) have been reported from the seeds. The plant contained major monomeric flavan-3-ol, catechin-3-glucose and epicatechin and the seeds possessed 4-chloro-1H-indole-3-*n*-methylacetamide, itaconic acid, arbutin, gentisic acid-5-*O*-[β-D-apiofuranosyl-(1→2)-β-D-xylopyranoside], and (6S,7Z,9R)-9-hydroxymegastig-ma-4,7-dien-3-one-9-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyran-oside (Tsopmo & Muir, 2010). In our previous study a new aromatic

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; $CDCl_3$, deuterated chloro-form; DMSO-d_6, deuterated dimethyl sulphoxide; TMS, tetramethylsilane.

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ester 3′-methyl–*n*-pentadecanyl benzoate along with β-sitosteryl *n*-octadec-9′-enoate, *n*-tetradecanyl linoleiate and *n*-octatriacosanoic acid were isolated from the seeds of lentil (Jameel, Ali, & Ali, 2014). In the present communication it has been aimed to characterize and evaluate novel antioxidant molecules from the seeds of *L. culinaris* of Delhi region which provides essential data and information.

2. Material and methods

2.1. General procedures

UV spectra were measured with a Lambda Bio 20 Spectrophotometer (Perkin Elmer, Rotkreuz, Switzerland) in methanol. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker ARX-Spectrometer (Rheinstetten, Baden-Wurttemberg, Germany), using $CDCl_3$ and DMSO-d_6 as solvents and TMS (Fluka analytical, Sigma–Aldrich, Zwijndrecht, Netherland) as an internal standard. Melting points were determined by a thermoelectrically heated Perfit melting point apparatus (Ambala, India) without correction. Infrared (IR) spectra were recorded using KBr pellets with a Jasco FT-IR-5000 Spectrometer (FTS 135, Kawloon, Hong Kong). Mass-spectrometric detection was carried out on ESI MS (Q-TOF-ESI) (Waters Corp., Manchester, UK), an electrospray-ionization (ESI) technique with positive ionization mode. Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60–120 mesh and solvents taken were purchased from Merck Specialties (E. Merck, Pvt. Ltd., New Delhi, India). Pre-coated aluminum TLC plates of silica gel 60 F_{254} (Merck, Darmstadt, Germany) were used to run and spots were visualized by exposure to iodine vapors, and UV radiations and spraying with anisaldehyde-sulfuric acid solution.

Folin–Ciocalteu reagent, DPPH radical, nitro blue tetrazolium (NBT), phenazine methosulfate, nicotinamide adenine dinucleotide, trichloro acetic acid (TCA), thiobarbituric acid (TBA), and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India).

2.2. Plant material

The seeds of *L. culinaris* were collected from the Herbal Garden of Jamia Hamdard, New Delhi and identified by Prof. Javed Ahmad, incharge of the Herbal garden. A specimen voucher of the drug was deposited in the Phytochemistry Research Laboratory, Jamia Hamdard with a reference number PRL-JH/2011/05.

2.3. Preparation of crude extract and isolation

The dried *L. culinaris* seeds (3.5 kg) were coarsely powdered sieved (60 mesh) and extracted with 5 L of methanol for 72 h in a Soxhlet extractor. The extract was dried to obtain a dark brown residue (232 g, yield 0.065%). The residue (100 g) was dissolved in a minimum amount of methanol and adsorbed on column grade silica gel (60–120 mesh) to obtain a slurry. The slurry was dried in air and chromatographed over a silica gel column loaded in petroleum ether. The column was eluted with petroleum ether–chloroform (1:1, *v*/*v*), chloroform and chloroform–methanol (99:1, 17:3 and 1:1, *v*/*v*) mixtures to isolate the compounds **1**–**6**.

2.3.1. n-Heptadecanyl oleate (**1**)

Elution with petroleum ether–chloroform (1:1, *v*/*v*) gave a pale yellow semisolid mass of **1**, 5.2 g (3.44% yield), R_f 0.2 (chloroform–methanol, 6:1, *v*/*v*), IR λ_{max} (KBr); 1721, 1617, 721 cm^{-1}; ESI MS *m*/*z* (rel. int.); 520 $[M]^+$ ($C_{35}H_{68}O_2$) (12.3), 281 (10.2), 255 (12.7), 239 (21.6).

2.3.2. β-Sitosterol (**2**)

Elution with chloroform furnished colorless crystals of **2**, recrystallized from chloroform–methanol (1:1, *v*/*v*), 510 mg (0.033% yield), R_f 0.7 (petroleum ether–chloroform, 1:1, *v*/*v*), m.p. 135–136 °C. IR λ_{max} (KBr); 3446, 1653 cm^{-1}; ESI MS *m*/*z* (rel. int.); 414 $[M]^+$ ($C_{29}H_{50}O$) (1.3).

2.3.3. β-Sitosteryl-3-(2′-n-eicosanyloxy)-benzoate (**3**)

Elution with chloroform–methanol (99:1, *v*/*v*) furnished a pale yellow sticky semisolid mass of **3**, purified from chloroform methanol (1:1, *v*/*v*), 430 mg (0.028% yield), R_f 0.6 (chloroform); UV λ_{max} (MeOH): 207, 275 nm (log ε 4.3, 1.8); IR λ_{max} (KBr): 2926, 2856, 1725, 1654, 1523, 1445, 1375, 1277, 1127, 1073, 980, 921, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.64 (1H, dd, *J* = 9.5, 2.8 Hz, H-3′), 7.46 (1H, dd, *J* = 8.7, 2.1 Hz, H-6′), 5.90 (1H, m, H-5′), 5.75 (1H, m, H-4′), 5.26 (1H, m, H-6), 4.27 (1H, brm, $w_{1/2}$ = 18.5 Hz, H-3α), 4.01 (2H, t, *J* = 6.6 Hz, H_2-1″), 0.92 (3H, brs, Me-19), 0.90 (3H, d, *J* = 6.9 Hz, Me-21), 0.84 (3H, d, *J* = 6.1 Hz, Me-26), 0.81 (3H, d, *J* = 6.3 Hz, Me-27), 0.79 (3H, d, *J* = 6.3 Hz, Me-29), 0.61 (3H, brs, Me-18), 1.07 (65H, m, 29× CH_2, 7× CH); ^{13}C NMR ($CDCl_3$): δ 36.12 (C-1), 31.51 (C-2), 71.74 (C-3), 41.20 (C-4), 140.76 (C-5), 121.63 (C-6), 31.91 (C-7), 31.67 (C-8), 50.06 (C-9), 37.28 (C-10), 21.09 (C-11), 39.79 (C-12), 42.31 (C-13), 56.76 (C-14), 24.32 (C-15), 28.25 (C-16), 56.06 (C-17), 12.03 (C-18), 19.42 (C-19), 35.59 (C-20), 18.82 (C-21), 33.95 (C-22), 26.11 (C-23), 45.82 (C-24), 29.15 (C-25), 19.87 (C-26), 19.20 (C-27), 23.10 (C-28), 11.89 (C-29), 139.21 (C-1′), 167.57 (C-2′), 132.40 (C-3′), 114.13 (C-4′), 128.85 (C-5′), 130.89 (C-6′), 173.19 (C-7′), 62.08 (C-1″), 33.81 (C-2″), 27.71 (C-3″), 27.19 (C-4″), 25.55 (C-5″), 24.83 (C-6″), 29.68 (C-7″), 29.68 (C-8″), 29.68 (C-9″), 29.68 (C-10″), 29.61 (C-11″), 29.56 (C-12″), 29.53 (C-13″), 29.50 (C-14″), 29.45 (C-15″), 29.34 (C-16″), 29.10 (C-17″), 28.94 (C-18″), 22.68 (C-19″), 14.16 (C-20″); ESI MS *m*/*z* (rel. int.): 814 $[M]^+$ ($C_{56}H_{94}O_3$) (1.5).

*2.3.3.1. Alkaline hydrolysis of compound **3**.* Compound **3** (25 mg) was heated with 70% aqueous ethanol (5 mL) and 1 M sodium hydroxide solution (2 mL) for 30 min. The reaction mixture was dried under vacuum and dissolved in petroleum ether to separate *n*-eicosanol, ESI MS 298 $[M]^+$ ($C_{20}H_{42}O$) (1.8%). The reaction mixture was dissolved in water and extracted with chloroform (3 × 5 mL) to separate the steroid. The chloroform extract was washed with water (2 × 5 mL), dried over anhydrous sodium sulfate and evaporated to produce β-sitosterol, m.p. 135–136 °C, co-TLC comparable. The aqueous solution after separation of the sterol was acidified with dil. HCl to pH 3 and re-extracted with chloroform to isolate salicylic acid, m.p. 157–159 °C, R_f 0.71 (ethyl acetate).

2.3.4. Oleyl-β-D-glucuranopyranoside (**4**)

Elution with chloroform–methanol (99:1, *v*/*v*) afforded a yellowish sticky semisolid mass of **4**, purified from chloroform, 995 mg, (0.065% yield), R_f 0.7 (chloroform), UV λ_{max} (MeOH): 222, 273 nm (log ε 5.1, 1.1). IR λ_{max} (KBr): 3415, 3381, 2908, 2847, 1725, 1705, 1449, 1365, 1164, 980, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.44 (1H, d, *J* = 7.0 Hz, H-1′), 5.39 (1H, m, H-9), 5.25 (1H, m, H-10), 4.31 (1H, m, H-5′), 4.15 (1H, m, H-2′), 4.10 (1H, m, H-3′), 3.60 (1H, m, H-4′), 2.52 (2H, t, *J* = 7.2 Hz, H_2-2), 2.20 (2H, m, H_2-8), 2.02 (2H, m, H_2-11), 1.61 (2H, m, CH_2), 1.25 (20H, brs, 10× CH_2), 0.83 (3H, t, *J* = 6.5 Hz, Me-18), ^{13}C NMR ($CDCl_3$): δ 173.32 (C-1), 39.33 (C-2), 32.75 (C-3), 32.14 (C-4), 31.87 (C-5), 29.06 (C-6), 29.22 (C-7), 37.19 (C-8), 133.01 (C-9), 130.21 (C-10), 33.99 (C-11), 29.31 (C-12), 29.41 (C-13), 27.92 (C-14), 25.43 (C-15), 24.70 (C-16), 22.63 (C-17), 14.07 (C-18), 102.06 (C-1′), 71.37 (C-2′), 68.05 (C-3′), 65.01 (C-4′), 73.21 (C-5′), 178.87 (C-6′). ESI MS *m*/*z* (rel. int.): 458 $[M]^+$ ($C_{24}H_{42}O_8$) (1.5), 281 (12.3).