Revista Brasileira de Farmacognosia 27 (2017) 488–494

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Comparative study of the hypocholesterolemic, antidiabetic effects of four agro-waste *Citrus* peels cultivars and their HPLC standardization

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ARTICLE INFO

Article history:
Received 20 July 2016
Accepted 2 January 2017
Available online 29 April 2017

Keywords:
Agro-waste *Citrus* peels
Anti-diabetic
HPLC quantification
Hypocholesterolemic
Pectin
Polymethoxyflavone

ABSTRACT

Citrus is an economically important fruit for Egypt, but its peel also is one of the major sources of agricultural waste. Due to its fermentation, this waste causes many economic and environmental problems. Therefore it is worthwhile to investigate ways to make use of this citrus waste generated by the juice industry. This study was aimed to explore the hypocholesterolemic, antidiabetic activities of four varieties of citrus peels agrowastes, to isolate the main flavonoids in the active fractions and to quantify them by HPLC method for nutraceutical purposes. All the tested samples of the agro-waste *Citrus* fruits peels showed significant decrease in cholesterol, triacylglyceride and glucose. The most decrease in cholesterol level was observed by mandarin peels aqueous homogenate and its hexane fraction (59.3% and 56.8%, respectively) reaching the same effect as the reference drug used (54.7%). Mostly, all samples decrease triacylglyceride (by 36%–80.6%) better than the reference drug used (by 35%), while, glucose was decreased (by 71.1%–82.8 and 68.6%–79.6%, respectively) mostly by the aqueous homogenates (except lime) and alcoholic extracts (except mandarin) of *Citrus* fruits peels better than the reference drug used (by 68.3%). All the isolated pectin, from the four cultivars, has significant effect on the three parameters. The comparative HPLC rapid quantification of nobiletin in the different by-product citrus varieties hexane fractions revealed that nobiletin is present in higher concentration in mandarin (10.14%) than the other species. Nobiletin and 4′,5,7,8-tetramethoxy flavone were isolated from mandarin peels hexane fraction by chromatographic fractionation. This is the first report of the comparative HPLC quantification of nobiletin and biological studies of different citrus peels species as agro-waste products. Based on these results, we suggest the possibility that *Citrus* fruits peels may be considered as an antidiabetic and hypocholesterolemic nutraceutical product.

Introduction

One of the most important popular threat factors for coronary heart disease, heart attack and stroke is the high cholesterol (AHA, 2014). Large amount of fruit peels are pitched as waste from fruit processing industry in spite of the well declared biological activities of these peels compared to other discarded portions. Among the fruits, *Citrus* fruits which its yield is approximated as 80 million tons per year are regarded as precious healthy diet since its nutrients boost haleness and guard against chronic disease (Gnanasaraswathi et al., 2014). Huge amounts of scraps are produced yearly, following juice manufacturing, from about one third of *Citrus* fruits (Li et al., 2006). Citrus peels, which constitute the main residue, contain more bioactive compounds than do juices (Bocco et al., 1998; Gorinstein et al., 2001) and are a suitable provenance of pectin (Sakai and Okushima, 1980). While citrus peels exhibit potent antioxidant, antimicrobial, anti-inflammatory activities (Murakami et al., 2000; Lin et al., 2011; Dhanavade et al., 2011) and have a reverse relationship with the coronary heart disease incidence by its potency in decreasing plasma cholesterol level (Bok et al., 1999; Wilcox et al., 2001; Whitman et al., 2005; Lee et al., 2011; Assini et al., 2013), pectin is useful in medical purpose, in which it aids in decreasing serum cholesterol level, dislodging heavy metal ions from the body, equilibrating blood pressure and assisting in weight reduction (Tang et al., 2011).

A plentiful source of polyhydroxyl flavonoids, such as hesperidin, neohesperidin and naringin, are the citrus peels which are

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http://dx.doi.org/10.1016/j.bjp.2017.01.010

also the unique source of polymethoxyflavones with high content such as nobiletin, tangeretin and sinesetin (Li et al., 2006; Londoño et al., 2010). In previously *in vivo* studies, lower doses of citrus polymethoxylated flavones (PMF) can decrease plasma cholesterol level more than that of flavanones (Morin et al., 2008) and modulated lipid metabolism in cells and animals (Lee et al., 2011). Two polymethoxylated citrus flavonoids, tangeretin and nobiletin, moderately inhibited both cholesterol (CH) and triacylglyceride (TG) synthesis, while weaker effects were reported by other PMF (*e.g.*, sinensetin) and non-PMF (*e.g.*, hesperetin and naringenin). Hence, attention should be paid for their proper extraction as potential compounds and check their suitability as therapeutics. This will increase the aggregate value of the industrial waste.

Our study was carried out on four agro-waste *Citrus* peels species cultivated in Egypt, after previously exploring their peels oil benefits (Abd-Elwahab et al., 2016), [mandarin (*Citrus reticulata* Blanco cv. Egyptian), sweet orange (*Citrus sinensis* (L.) Osbeck cv. Olinda Valencia), white grapefruit (*C. paradisi* Macfad. cv. Duncan) and lime (*C. paradisi aurantiifolia* (Christm.) Swingle cv. Mexican)], Rutaceae, to evaluate and compare their hypocholesterolemic and anti-diabetic activities as agro-waste products, to isolate the main flavonoids in the active fractions and to quantify them by HPLC method for nutraceutical purposes to facilitate the conversion of this waste into high value-added products, thus allowing it to be a recycled component of functional food material.

Material and methods

Plant material

Samples of the fresh fully mature ripe citrus fruit peels [mandarin (*Citrus reticulata* Blanco cv. Egyptian), sweet orange (*C. sinensis* (L.) Osbeck cv. Olinda Valencia), white grapefruit (*C. paradisi* Macfad. cv. Duncan) and lime (*C. aurantiifolia* Swingle cv. Mexican)], Rutaceae, were identified by Citrus department, Horticultural Institute, Ministry of Agriculture, Giza, Egypt and voucher specimens numbers 14-7-2016-I, 14-7-2016-II, 14-7-2016-IV and 14-7-2016-III, respectively, were deposited at the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt. The material were collected in February 2011 (for sweet orange), September 2011 (for lime), 2nd half of December 2011 (for grapefruit) and 2nd half of January 2012 (for mandarin), from the private orchard of El-Mazloom company for horticulture production at 78 km Cairo-Ismailia road.

Preparation of extracts and isolation of pectin

Preparation of a citrus peels aqueous homogenates: the aqueous homogenates were prepared by mixing 1.5 g of fresh peel of each species with 100 ml distilled water in a blender, stored in bottle and kept in refrigerator (2–5 °C) until used.

Preparation of a citrus peels alcoholic extracts: the alcoholic extracts were prepared by percolating 200 g of fresh peel with 80% methanol (600 ml), filtered off and the resulting extracts were evaporated under reduced pressure.

Preparation of a *Citrus* peels hexane extracts: part of the previously prepared alcoholic extract residue (10 g) for each Citrus fruits peels were suspended separately in distilled water (30 ml), then extracted with *n*-hexane and the resulting hexane extracts were evaporated under reduced pressure.

Isolation of pectin from citrus peels: each species of fresh citrus peels (50 g) was boiled with known volume of distilled water (100 ml), decanted, cooled to room temperature (25 °C) and then four volumes of absolute ethanol (400 ml) was added to precipitate the pectin, kept in refrigerator (2–5 °C) for two days and then filtered to obtain pectin which is freeze dried and stored in desiccators until used.

Citrus peels alcoholic and hexane extracts residues and the isolated pectins (1.5 g of each) were mixed with 100 ml distilled water (1.5%), stored in bottle and kept in refrigerator (2–5 °C) until used.

Materials

For biological study

Pure cholesterol powder analytical grade (C75209) and bile salts powder (48305) were purchased from Sigma Chemical Co., St. Louis, USA. Metformin (Cidophage®): Chemical Industries Development Co. (CID Co.), Giza, Egypt, as an anti-diabetic reference drug. Atorvastatin 5 mg (Lipitor®): Pfizer Company, Cairo, Egypt, as a hypolipidemic reference drug.

For chromatographic study

Silica gel 60 (89943 Fluka) for column chromatography and precoated silica gel plates 60 F254 for TLC were obtained from Sigma-Aldrich Co. LLC and Merck Millipore Corporation, Germany, respectively. Acetonitrile (34860), methanol (34860) and phosphoric acid (79606) of HPLC grade were purchased from Sigma Chemical Co., St. Louis, USA. ^{1}H-(300 MHz) NMR spectra were recorded on Varian Mercury apparatus at 25 °C using TMS as an internal standard and chemical shifts were given in δ values.

Isolation of polymethoxyflavones

The air dried powdered mandarin peels (850 g) were macerated in 75% alcohol (2× 3 l) at room temperature and the alcohol extract was evaporated under vacuum, to obtain a yellowish brown residue (233 g). This residue was suspended in distilled water (500 ml) and then fractionated with hexane (250× 3 ml), to yield 5 g residue after evaporation under reduced pressure. The latter was chromatographed on silica gel column; gradient elution was carried out using hexane containing 10% stepwise increments of acetone till 100% acetone. Fractions, 100 ml each, were collected to yield 40 fractions and subjected to TLC on pre-coated silica gel plates, using solvent systems chloroform: methanol (98:2). Fractions (17–20) eluted with hexane:acetone (6:4) showed a major spot that appears yellow in UV at $\lambda_{365\,nm}$ and gave a yellow color with *p*-anisaldehyde spray reagent, ammonia and aluminum chloride. These fractions were pooled together and the solvent was evaporated under reduced pressure to yield compound **1** as a yellow powder (1.5 g). Fractions (29–32) eluted with hexane: acetone (3:7) showed a major spot that appears yellow in UV at $\lambda_{365\,nm}$ and gave a yellow color with *p*-anisaldehyde spray reagent, ammonia and aluminum chloride. These fractions were pooled together and the solvent was evaporated under reduced pressure to yield compound **2** as a yellow powder (0.75 g).

HPLC analysis

HPLC analyses were performed using Agient Technologies 1200 series; consisting of G1322A Degasser (serial No. JP94172767), G1311A Quat pump (serial No. DE62972789), G1314B VWD (serial No. DE71365992) and G1328B Man. inj. (serial No. DE60561522). The wavelength used for the quantification of the flavanones glycosides with the UV detector was 325 nm. The chromatographic separation was carried out on LiChrosher(R) 100 RP-18 endcapped (5 μm) column. LiChroCART(R) 250-4 HPLC-Cartridge, Agilent Technologies, Part No. 79925ODE-584, Cartridge No. 031399 and Sorbent Lot No. L010077333.

Table 1
Ingredient composition of the diet fed to rats.

Standard laboratory diet	Percentage (%)
Vitamin mixture	1
Mineral mixture	4
Corn oil	10
Sucrose	20
Cellulose	0.2
Starch	54.3
Casein (95% pure)	10.5

The end-capped column was held at 37 °C and the flow rate was set at 1 ml/min. The chosen mobile phase was as that used by Sun et al. (2010) but with little modification as following: the mobile phase consisted of two solvents; 90% phosphoric acid (0.3%) (A) and 10% acetonitrile (B). The solvent gradient in volume ratios was as follows: 10–40% B over 1 min. The solvent gradient was maintained at 40% B for 1 min, increased to 45% B over 18 min, and to 100% B over 2 min, then maintained at 100% B for 7 min. The injection volume was 20 μl. Analyses were performed at least three times and only mean values were reported.

The four tested *Citrus* peels hexane extracts were prepared at a concentration of 2 mg/ml and filtered on Ekicrodisc® 3.Acro LC (3 mm diameter, 0.45 μm, HPLC certified, LOT No. A10422144 P/N E031) before use. The isolated standard nobiletin was prepared at a stock concentration of 500 μg/ml. Calibration standard sample was prepared by appropriate dilutions with methanol from the stock solutions and filtered on Ekicrodisc® 3.Acro LC before use. Calibration curves were obtained by plotting the peak area of the standard *versus* its concentrations ($0.92 < R^2 < 0.99$). Concentrations of the nobiletin in samples were determined by application of the obtained standard curve.

Experimental animals and protocols

Male Wistar strain rats weighing 150–160 g, aged three months, utilized for assessment of the different pharmacological effects, were supplied from the Research Institute of Ophthalmology. They were individually housed six per cage (320 cm × 180 cm × 160 cm) under standardized temperature (25–28 °C), humidity (50–60%) and light (12 h light/dark cycles with the light on at 7 a.m. and had free access to tap water and food pellets conditions. They were fed the standard laboratory diet as shown in Table 1.

Hypercholesterolemia was induced by feeding the male Wistar rats with standard laboratory diet mixed with 1% cholesterol and 0.25% bile salts powders from the diet weight (Berrougui et al., 2003; Owens, 2006). Blood samples were taken at the eighth week of experiment and then centrifuged at 715 × *g* force for 10 min for biochemical analyses of plasma parameters. The clear plasma was separated and divided into three portions to measure the plasma glucose, triacylglyceride and cholesterol levels by using specific kits. The separated plasma was stored at −80 °C until analysis. This was carried out according to Odetola et al. (2006) and Owens (2006). Untreated model (high fat diet) and the negative control: received daily 0.1 ml of distilled water orally beside standard laboratory diet.

Treated model groups (16 groups): administered 0.1 ml of the corresponding extract or pectin or aqueous homogenate orally beside standard laboratory diet.

Biochemical plasma analyses

Plasma glucose, TG and total cholesterol levels were determined by enzymatic methods using commercial assay kits (glucose kits, triacylglyceride kits and total cholesterol kits) according to the manufacturer's protocols, purchased from Biodiagnostic Company (Egypt). Specto UV–vis Double Beam PC, 8 scanning auto cell, UVD-3200 (LABOMED, Inc.) was used for evaluation of anti-diabetic and hypocholesterolemic activities by measurement of the color intensity at 515–520 nm.

Statistical analysis

All the data were expressed as the mean ± standard error of the mean (SEM). The statistical significance of differences between the mean values for the treatment groups was analyzed by one-way analysis of variance (ANOVA) followed by *Post Hoc* test and Bonferroni (Snedecor and Cochran, 1982) using the SPSS software (SPSS Inc., Chicago, USA). A value of $p < 0.05$ was considered statistically significant for analysis. Correlation analysis was carried out and R-square values were reported.

Results and discussion

The percentage yield of peels and pectin obtained from different agro-waste peels

Both white grapefruit and mandarin have the highest percentage yield of fresh peels, recording 25.81% and 23.31% respectively, while lower yield was observed for the other two remaining tested *Citrus* fruits, sweet orange and lime, recording the same result 18%. Upon drying the peels, all the *Citrus* fruits show nearby results; 5.80% in white grapefruit to 7.19% in lime fruits. All the isolated pectin from the tested agro-waste peels occurs as a white powder. Sweet orange have the highest content of pectin (21.33%) followed by lime (19.7%) and grapefruit (11.66%), while mandarin have the lowest pectin contents (9.14%). Our results showed a variation from the previously reported ones, Rouse and Crandall (1976) reported 8.15% pectin yield in fresh ground Valencia orange peels and 6.35% in Duncan grapefruit peels extracted with nitric acid. Both Liu et al. (2001) and Sakai and Okushima (1980) recorded 3.68% and 0.4% for Marrs and Navel orange peel, respectively, and 2.99% and 0.2% for Marsh and Navel grapefruit peel, respectively, prepared by classical hot acid procedure and water extraction. While the pectin yields of sweet orange and mandarin extracted by the addition of sulphuric acid was 43.7% and 37.3%, respectively (Wang et al., 2008). Therefore, the pectin yield depends not only on the species and/or cultivars but also on the method used for preparation.

Identification of the isolated Citrus *flavonoid*

The structure of the isolated compound (**1**) was identified as nobiletin [2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-4*H*-1-benzopyran-4-one] by comparing its physical and spectral data with the reported ones (Dandan et al., 2007; Johann et al., 2007). Compound **1**: colorless needles; $C_{21}H_{22}O_8$; EI/MS *m/z* (70 ev) 402 $[M]^+$, 387 $[M\text{-}CH_3]^+$; mp. 137–138 °C; IR γ_{max} (KBr cm^{-1}): 2943.7, 2839.7, 1645.9, 1592.2, 1520.1, 1462.5, 1370.7, 1277.7, 1016.2, 840.7, 803.4; ^{1}H NMR (300 Hz, DMSO) δ: 7.63 (1H, *dd*, *J* = 8.7 and 2.1 Hz, H-6′), 7.53 (1H, *d*, *J* = 2.1 Hz, H-2′), 7.14 (1H, *d*, *J* = 8.7 Hz, H-5′), 6.83 (1H, *s*, H-3), 4.02, 3.97 (each 3H, *s*, OMe), 3.88, 3.85, 3.84, 3.78 (12H, overlapped, 4 × OMe).

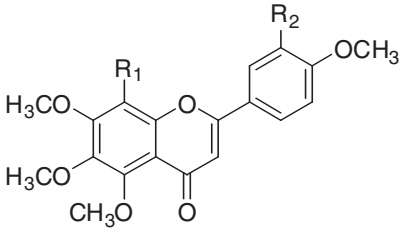
The structure of the isolated compound (**2**) was identified as 4′,5,7,8-tetramethoxyflavone by comparing its physical and spectral data with the reported one (Uckoo et al., 2012). Compound **2**: colorless needles; $C_{19}H_{18}O_6$; EI/MS *m/z* (70 ev) 342 $[M]^+$, 327 $[M\text{-}CH_3]^+$, 311 $[M\text{-}OCH_3]^+$; mp. 141–142 °C; ^{1}H NMR (300 Hz, $CDCl_3$) δ: 7.88 (1H, *d*, *J* = 8.7 Hz, H-2′ & 6′), 7.02 (1H, *d*, *J* = 8.4 Hz, H–3′ & 5′), 6.62 (1H, *s*, H-3), 6.44 (1H, *s*, H-8), 6.62 (1H, *s*, H-3), 4.02, 3.99, 3.96, 3.89 (each 3H, *s*, OMe). ^{13}C NMR (75 Hz, $CDCl_3$) δ: 176.37 (C-4), 162.37 (C-2), 160.28 (C-4′), 156.86 (C-9), 156.25 (C-7), 151.69 (C-5), 130.63 (C-8), 127.90 (C-6′), 127.66 (C-2′), 123.77 (C-1′), 114.84

Table 2
Mean concentraion and percentage of the isolated standard nobelitin in the four tested hexane citrus peels extracts.

Tested hexane *Citrus* peel (2 mg/ml)	Mean concentraion of nobelitin[a] (μg/ml) ± SE	Percentage of nobelitin (%)
Mandarin	202.91 ± 3.63	10.14
Sweet orange	73.15 ± 3.08	3.6
Grapefruit	18.13 ± 0.33	0.9
Lime	0.09 ± 0.34	0.0045

[a] Mean concentraion of nobelitin is based on the average of three determinations ± standard error.

(C-5′), 114.49 (C-3′), 108.52 (C-10), 106.31 (C-3), 93.44 (C-6), 61.02 (C of OMe at C8), 60.66 (C of OMe at C7), 55.90 (C of OMe at C5), 55.05 (C of OMe at C4′).



1 R_1=R_2=OCH_3
2 R_1=R_2=H

HPLC results

In the current study, the chromatographic profiles of different agro-waste citrus peels revealed that madarain hexane peel extact has the highest concentration of the isolated nobiletin (202.91 μg/ml ± 3.63; 10.14%), nearly more than twice that in sweet orange (73.15 μg/ml ± 3.08; 3.6%) and ten times as that in grapefruit (18.13 μg/ml ± 0.33; 0.9%). However, lime is nearly depleted of it (Table 2; Fig. 1). This is the first HPLC study comparing the percentage of nobiletin in these four new Egyptian citrus varieties.

The anti-diabetic and hypocholesterolemic effects of the different agro-waste citrus peels

In general, all the tested samples (the alcoholic extracts, hexane extracts, isolated pectins and the aqueous homogenates) of the four tested agro-waste citrus peels had shown significant decrease in all the tested parameters (cholesterol, triacylglyceride and glucose) (Table 3; Fig. 2).

The alcoholic extract, hexane extract and aqueous homogenate of mandarin peels show the highest decrease (by 48.9%, 56.8% and 59.3%) in cholesterol level (77.5 ± 13.3, 65.5 ± 20.6 and 61.8 ± 17.6 mg/dl, respectively) equivalent to that of atorvastatin reference drug (68.7 ± 1.09 mg/dl [54.7%]). Mandarin alcohol peel extract was previously reported to improve blood cholesterol profile in dose dependant manner (Adelina et al., 2008). The presence of the main bioactive components as flavanones (hesperetin, naringenin), flavone glycosides (hesperidin, naringin) and methoxylated flavones (PMF) in *Citrus* fruits (Manthey and Guthrie, 2002) could clarify the hypocholesterolemic impacts of both alcoholic extracts and aqueous homogenates of the four tested agro-waste citrus peels as the existence of hesperidin and naringin, and their aglycones hesperetin and naringenin, have been accounted for lowering plasma and hepatic cholesterol and triacylglycerol by inhibiting these hepatic enzymes in experimental animals (Kim et al., 2003)

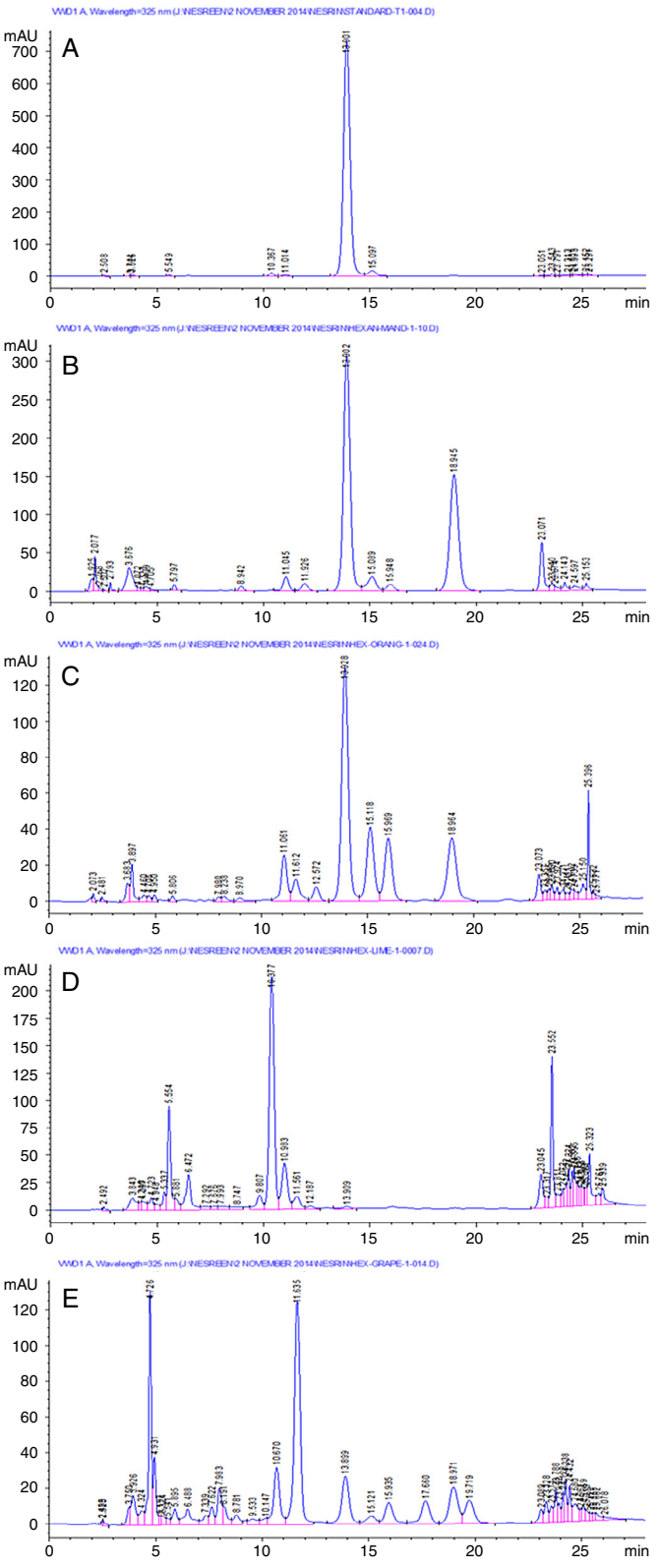


Fig. 1. HPLC chromatogram at 325 nm of the four tested citrus peels hexane extracts (2 mg/ml) against the isolated standard nobiletin (0.5 mg/ml). [A, nobiletin; B, mandarin hexane extract; C, orange hexane extract; D, lime hexane extract; E, grapefruit hexane extract].

Table 3
The mean values ($M \pm SE$) of the plasma cholesterol, triacylglyceride and glucose levels (mg/dl) of the control and model groups treated with alcoholic extracts, *n*-hexane extracts, isolated pectin and aqueous homogenates of the tested Citrus peels.

Groups[a]	Mean ± SE of		
	Cholesterol (mg/dl)[b]	Triacylglyceride (mg/dl)[b]	Glucose (mg/dl)[b]
Negative control	64.9 ± 0.80	72.7 ± 2.98	83.2 ± 1.43
Model treated with reference drug[c]	68.7 ± 1.09	153.3 ± 1.95	86.7 ± 1.34
Model of hypercholesterolemic non treated rats	151.9 ± 27.1	236 ± 36.0	274.2 ± 42.6
Model treated with peel alcoholic extract of			
Mandarin	77.5 ± 13.3	69.9 ± 24.8[f]	149.8 ± 24.8[f]
Sweet orange	125.9 ± 20.7	83.3 ± 18.5[f]	55.9 ± 17.5[f]
White grapefruit	153.1 ± 14.5	75.5 ± 28.0[f]	86.0 ± 13.8[f]
Lime	211.6 ± 14.1[d,e]	45.7 ± 16.3[e,f]	56.4 ± 15.7[f]
Model treated with peel n-hexane extract of			
Mandarin	65.5 ± 20.6	53.9 ± 7.3[e,f]	153.4 ± 18.7[f]
Sweet orange	78.6 ± 21.3	63.8 ± 22.9[f]	111.6 ± 17.6[f]
White grapefruit	109.8 ± 22.8	63.6 ± 16.5[f]	91.0 ± 13.2[f]
Lime	73.2 ± 7.3	83.5 ± 7.9 [f]	124.0 ± 13.6[f]
Model treated with peel pectin of			
Mandarin	98.6 ± 29.8	50.3 ± 20.0[e,f]	189.0 ± 24.8[d]
Sweet orange	82.6 ± 24.1	61.6 ± 16.8[f]	151.9 ± 7.8[f]
White grapefruit	98.5 ± 4.5	116.4 ± 12.4[f]	168.4 ± 6.5[f]
Lime	75.4 ± 18.2	73.3 ± 18.2[f]	202.7 ± 11.4[d,e]
Model treated with peel aqueous homogenate of			
Mandarin	61.8 ± 17.6	150.9 ± 8.6[f]	54.5 ± 7.9[f]
Sweet orange	80.6 ± 29.8	165.9 ± 141[d]	46.9 ± 9.5[f]
White grapefruit	80.4 ± 17.6	117.4 ± 8.8[f]	79.2 ± 27.4[f]
Lime	81.4 ± 11.0	107.2 ± 5.6[f]	160.5 ± 14.8[f]

[a] Number of male Wistar strain rats (150–160 g, aged three months) in each group is six.
[b] Concentration of each parameter is based on average of four determinations ± standard error of mean for group.
[c] Reference drug for glucose is Metformin (20 mg/kg b.wt). Reference drug for cholesterol and triacylglyceride is atorvastatin (5 mg/kg b.wt).
[d] Value is significant difference when compared to the negative control group at $p < 0.05$.
[e] Value is significant difference when compared to the model treated with reference drug at $p < 0.05$.
[f] Value is significant difference when compared to the untreated hypercholesterolemia model (high fat diet) at $p < 0.05$.

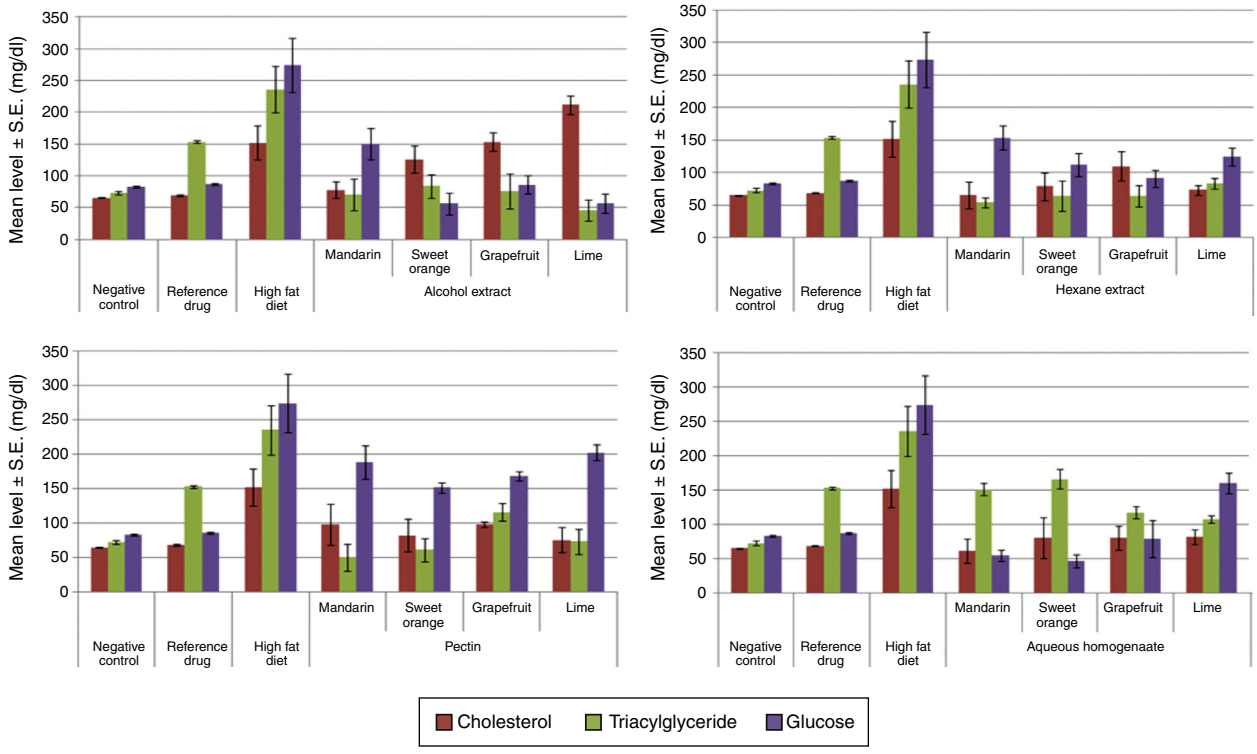


Fig. 2. Histogram representing the effect of the alcohol extracts, hexane extracts isolated pectin and aqueous homogenates of the four tested citrus peels on the plasma cholesterol, triacylglyceride and glucose levels in hypercholesterolemic rats. Reference drug for glucose is Metformin (20 mg/kg b.wt), for cholesterol and triacylglyceride is atorvastatin (5 mg/kg b.wt).

and regulating the fatty acid and cholesterol metabolism (Jung et al., 2006).

Mostly, all the tested samples decrease TG (by 36.0–80.6%) (ranging from 150.9 ± 8.68 to 45.7 ± 16.35 mg/dl) better than the atorvastatin reference drug used (153.3 ± 1.95 mg/dl [35.0%]), while, glucose was decreased mostly by the aqueous homogenates (except lime) (by 71.1–82.8%) (ranging from 79.2 ± 27.4 to 46.9 ± 9.58 mg/dl) and alcoholic extracts (except mandarin) by (68.6–79.6%) (ranging from 86.0 ± 13.8 to 55.9 ± 17.5 mg/dl) of *Citrus* fruits peels better than the metformin reference drug used (86.7 ± 1.34 mg/dl [68.3%]).

Thus the observed anti-diabetic and hypocholesterolemic effects of the different agro-waste citrus peels extracts could be attributed to the presence of nobiletin (Tsutsumi et al., 2014) with the obvious decrease in cholesterol level in case of mandarin due to its highest percentage of nobiletin as revealed from HPLC analysis (Table 2). Previous studies on *Citrus* peels reported similar effects (Kurowska and Manthey, 2004; Nagata et al., 2010; Lee et al., 2014; Tsutsumi et al., 2014) and contributed them to the presence of PMF like nobiletin which stimulates lipolysis in differentiated adipocytes, attenuated dyslipidemia through a reduction in VLDL-triacylglyceride secretion, prevented hepatic triacylglyceride accumulation, enhanced fatty acid B-oxidation (Mulvihill et al., 2011).

All the isolated pectin, from the four cultivars, has significant effect on the three tested parameters, with more pronounced decrease on triacylglyceride by 50.7–78.7% (ranging from 116.4 ± 12.4 to 50.3 ± 20.0 mg/dl) and little decrease on both cholesterol and glucose by 30–50.3% (ranging from 98.6 ± 29.8 to 75.4 ± 18.2 mg/dl) and 26–44.6% (ranging from 202.7 ± 11.4 to 151.9 ± 7.85 mg/dl), respectively. This was explained by the previously reported data that the viscosity-enhancing and gel forming properties of pectin could delay gastric emptying and possibly reduce the absorption rate in small intestine (de Escalada Pla et al., 2007). Besides, pectin as a kind of soluble dietary fiber, lowers blood cholesterol levels and LDL cholesterol fraction without changing HDL cholesterol (Liu et al., 2006; Shaha et al., 2013).

Since the aqueous homogenate is rich of both pectin and flavanoids (PMF), this could explain its well-pronounced effect in lowering cholesterol levels *via* modulating hepatic HMG-CoA levels, possibly by binding bile acids and increasing the turnover rate of blood and liver cholesterol (Marounek et al., 2007).

Conclusion

This is the first report about comparing positive effect of four new Egyptian varieties of agro-waste citrus peels extracts and their pectin on high-fat diet rats. The observed hypocholesterolemic and hypotriglycermic effect of the tested samples is directly proportional to their content of nobiletin. Other constituents rather than PMF also participate in the observed biological activities as lime exhibited certain activities although HPLC study revealed its depletion of these compounds. The aqueous homogenates of these agro-waste citrus peels can be used as anticholesterolemic and anti-diabetic drugs to save time and chemical consumption during extraction of these agro-waste products.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contribution

NMF (PhD student) contributed in collecting the plant material, preparation of all extracts, running the laboratory work, help in biological studies, performing the HPLC analysis, statistical analysis of the data and constructing the manuscript. AE contributed in performing the biological study. ARA contributed in revising the manuscript. SA, NE and MYM contributed in suggesting the point of the research and supervising the work. All the authors have read the final manuscript and approved its submission.

Conflicts of interest

The authors declare no conflicts of interest.

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