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# Chemical and biological assessment of high value pharmaceutical raw material from Egyptian orange peel

Meselhy R. Meselhy[1], Shahira M. Ezzat[1,2], Dalia El-Sheikh Ali[3], Mohamed El-Adl[4], Reham A. El-Shafei[5], Marwa M. Abd El-Aziz[6] & Essam Abdel-Sattar[1]*

[1]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt
[2]Pharmacognosy Department, Faculty of Pharmacy, October University for Modern Science and Arts (MSA), 6th of October, 12566 Egypt
[3]Department of Pharmacognosy, Faculty of Pharmacy, Pharos University in Alexandria, Alexandria, Egypt
[4]Department of Biochemistry; [5]Department of Pharmacology, Faculty of veterinary, Mansoura University, Mansoura, Egypt
[6]The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt

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In Egypt, orange juice industries generate huge amount of waste peel that could be a source of raw materials with high value and economic return. Here, we explored a better model for waste management of citrus processing waste in Egypt by developing an ecofriendly method for preparation of value added materials. A high grade pectin (HGP) was obtained from the crude acidic water extract of the peel after passing through Diaion HP20 column. The HGP showed potent antidiabetic activity at lower dose than those reported in literature. It possessed significant effect on blood glucose (BG) level, as well as parameters relevant to liver and kidney functions in streptozotocin (STZ) induced diabetic rats. On the other hand, the essential oil (EO) prepared by cold press showed the highest yield (0.72% w/w) and it is the most applicable method of isolate orange oil on pilot scale. EO showed significant antimicrobial activity against the tested food borne pathogens. In conclusion, high value materials — HGP and EO were prepared on pilot scale from the waste orange peel. While the HGP can be included in food supplement for diabetic patients, EO can be used as a natural food preservative.

**Keywords**: Antihyperglycemic, Antimicrobial activity, Pectin, Sweet orange, Waste utilization

Citrus is considered as one of the world's major fruit cros produced worldwide with annual production exceeding 124 million tons[1]. The Mediterranean countries, Spain, Italy, Egypt and Turkey, are the major producers of Citrus fruits, providing of 20% of global production. In this view, Citrus fruits are of high economic value to Egypt due to their multiple uses. Egypt is among the top 10 orange producers in the world. It produces more than 30% of total Egyptian fruits and 65% of total Citrus produced in Egypt. At present, the citrus cultivation has increased rapidly to 204 hectares, about 29% of total area for fruit cultivation (700,854 hectares), producing about 4.27 million metric tons[2]. Around 46% of the orange produced in Egypt is consumed fresh, 51% is exported and 3% is used for processing[3]. Fresh consumption and processing of the fruit generate large volumes of wet wastes, particularly the peel that represents about 30-40% of the fruit[1,4]. Most of the waste is currently used as cattle feed with poor economic return. However, these wastes have potential to be valorized as a source of high value materials for pharmaceutical and food applications of high economic return. Several research studies revealed that orange peel is a valuable raw material for the production of various useful products such as pectin, carotenoids and flavonoids, oils and perfumes, ethanol and citric acid[5-7]. Utilizing orange peel for an extracellular enzymateic complex was produced having a potent macerating activity (pectine lyase) by *Rhizopus oryzae* in solid state culture[8].

Though Egypt is ranked first globally in in orange export in 2020, surpassing the world leader Spain, and it shows negative trade balance as far as pectin import/export business (typical level of pectin in orange peel is >20%, though pectin used locally in different industries is exclusively imported). Production of pectin from orange peel involves hydrolysis of insoluble protopectin into soluble pectins and then leaching them out of peel tissues. Generally, boiling water was used as the simplest and oldest method for releasing the pectic substances from

*Correspondence:
E-Mail: abdelsattar@yahoo.com; essam.abdelsattar@pharma.cu.edu.eg

the tissues but have limited yield[9] and also extraction using boiling water containing various agents (including acid or base) has been reported. While the base-dependent extraction process yields pectin of low degree of esterification (low DE pectin. DE, Degree of esterification) as a result of saponification of the ester groups[10], the acid extraction process generally yields pectin of high degree of esterification (high DE pectin, 50% or greater), approximately equal to the naturally occurring DE. New research has resulted in the development of eco-friendly extraction processes such as microwave assisted extraction, enzymatic extraction[11], supercritical water extraction and ultra-sound extraction. Nevertheless, none of the methods extract all the pectin without causing some degradation. In addition, these methods still relatively expensive and do not have industrial significance when compared with the chemical route using mineral acids[12]. Experimentally, the most commonly used methods for the extraction of pectin include direct boiling and microwave heating[13,14] while industrial production of pectin rely primarily on direct extraction with hot dilute mineral acids as it is simple to construct and operate at relatively minimum cost.

In the present study, we explored acid extraction for making crude pectin from orange peel, and further to produce high-grade pectin (HGP) by filteration through microporous resin (Diaion HP-20) column to purify the crude pectin. We have also checked the fresh and dry orange peels as a potential sournce of the essential oil (EO) — orange oil adopting cold press and the hydro-distillation methods. In addition, we assessed the biological activities of HGP and the oil samples.

## Materials and Methods

### General and chemicals

Streptozotocin (STZ), ciprofloxacin (antibacterial agent) and amphotericin B (antifungal) were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). Metformin was obtained from Chemical Industry Development Co. (CID), Giza, Egypt, and was used as a reference antidiabetic drug. Transaminase kits (AST and ALT), creatinine, urea, cholesterol, triglyceride, ALP, albumin, MDA and globulin were purchased from Biodiagnostic for diagnostic reagents (Dokki, Giza, Egypt), and the kit for insulin was purchased from ALPCO Diagnostics (USA). Solvents used in this study were of analytical grade and purchased from local market.

### Plant materials

Samples of the fresh peels of Valencia orange [*Citrus sinensis* (L.) Osbeck, (Fam. Rutaceae], left after juice extraction were obtained from El-Marwa Food Industry (6$^{th}$ of October city, Egypt) in March 2017. The fruits were kindly authenticated by Dr. Masoud M.R.M., Horticulture Technology Department, Food Technology Research Institute, Agriculture Research Centre (ARC), Algamaa street, Giza , Egypt . A voucher specimen of the dried peels (No. 30.3.16.1) was deposited at the herbarium of the department of Pharmacognosy, Faculty of Pharmacy, Cairo University. The fresh peels were subjected to drying in an oven using hot air flow (@ 70°C for first 3 h then adjusted to 50°C for additional 3 h) at the ARC.

### Preparation of Essential oil

Three essential oil samples were prepared, EO-1 was prepared from 150 g of the fresh peel by cold press method[15], EO-2 was prepared from 250 g of the fresh peel by hydro-distillation for 5 h, while EO-3 was prepared from 150 g of the dried peels by hydro-distillation for 30 min. The hydro-distillation was performed according to the procedure described in the Egyptian Pharmacopeia[16]. The prepared essential oils were dried over anhydrous sodium sulfate to give 1.08, 0.5, 0.9 g of EO-1, EO-2 and EO-3, respectively. The oil samples were stored at −4°C until further analysis.

### GC/MS analysis of essential oils

Samples were analyzed according to the reported method by Javed *et al*.[17] using Shimadzu GCMS-QP2010 (Tokyo, Japan) equipped with Rtx-5MS fused bonded column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) (Restek, USA) and equipped with a split–splitless injector. The capillary column was directly coupled to a quadrupole mass spectrometer (SSQ 7000; Thermo-Finnigan, Bremen, Germany). The initial column temperature was kept at 45°C for 2 min (isothermal) and programmed to 300°C at a rate of 5°C/min, and kept constant at 300°C for 5 min (isothermal). Injector temperature was 250°C. Helium carrier gas flow rate was 1.41 mL/min. All the mass spectra were recorded applying the following condition: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 200°C. Diluted samples (1% v/v hexane) were injected with split mode (split ratio 1: 15).

**Preparation of pectin**

*Conventional method for preparation of pectin*

In this method, pectin sample was prepared according to the procedure reported by Kurita *et al*.[18]. Briefly, 100 g of powdered peel was added to boiling water with stirring for 30 min, then filtered while hot using muslin. The wet marc was pressed and extracted twice with 2.5 L of acidified boiling water (pH 1.6 with HCl) for 60 min and filtered. The marc was pressed and the combined filtrate (5 L) was cooled down to around 50°C and this filtrate was concentrated to 1500 mL under reduced pressure and 1.5 volumes of ethanol 95% was added and filtered. The precipitate was dried in oven at 50°C, and keep in desiccator until constant weight (19 g).

*Preparation of high grade pectin* (*HGP*)

Preparation of purified pectin samples was carried out according to the procedure reported earlier[16] with some modification. Briefly, 100 g of powdered peel was added to boiling water with stirring for 30 min, then filtered while hot using muslin. The wet marc was pressed and extracted two times with 2.5 L of acidified boiling water (pH 1.6 with HCl) for 60 min and filtered. The marc was pressed and the combined filtrate (5 L) was cooled down to around 50°C and this filtrate was applied to a column of Diaion HP20 (180 g, 46 × 3.5 cm). The column was eluted with distilled water (1.2 L). The combined eluates were concentrated to 1500 mL under reduced pressure and 1.5 volumes of ethanol 95% were added and filtered. The precipitate was dried in oven at 50°C, and keep in desiccator until constant weight 20.9 g.

*Analysis of pectin*

Pectin equivalent weight, methoxyl content, degree of esterification, anhydrouronic acid and were determined according to procedure reported by Khamsucharit *et al*.[19], in addition, Fourier transform infrared (FT-IR) spectroscopy analysis (FTIR, Shimadzu- IR-435, Kyoto, Japan) was performed using KBr pellets.

**Antimicrobial activity**

*Microorganisms*

Selected strains of bacteria and fungi were supplied by the Regional Center for Mycology and biotechnology, Faculty of Pharmacy, Al-Azhar University, Egypt. Gram positive bacterial strains: *Staphylococcus aureus* (RCMB 010027), *Enterococcus faecalis* (RCMB 01001 54-2) and *Listeria monocytogenes* (RCMB 01002 86-3). Gram negative bacterial strains: *Escherichia coli* (RCMB 01002 52-6), *Vibrio cholerae* (RCMB 01002 68-4), *Klebsiella pneumonia* (RCMB 01002 23-5), *Pseudomonas aeruginosa* (RCMB 01002 43-5), *Salmonella typhi* (RCMB 01002 15-4). Fungi: *Candida albicans* (RCMB 05036), *Aspergillus flavus* (RCMB 02768), *Penicillium islandicum* (RCMB 01938).

*Testing of antimicrobial activity of essential oil*

Antimicrobial activity of the essential oils was studied against number of bacterial and fungal strains. The samples were dissolved in DMSO at a concentration of 1.0 mg/mL and their antimicrobial activity was determined according to Kirby-Bauer disc diffusion method[20]. DMSO (100 µL) was used as negative control, ciprofloxacin (1.0 mg/mL) was used as positive control in case of gram positive and gram negative bacteria and amphotericin B (1.0 mg/mL) was used as positive control in case of fungi. Zones of inhibition were measured and diameters more than 5 mm were considered as a sign of inhibitory effect. Zone inhibition diameter (mm) and potency percentage of the tested samples relative to the standard drugs. The minimum inhibitory concentration (MIC) was also determined by Kirby-Bauer disc diffusion method. The lowest concentration of each extract or control drug showing a clear zone of inhibition was taken as MIC.

**Testing of the antidiabetic activity of HGP**

The antidiabetic activity was assessed according to the method reported by Chigurupati *et al*.[21] with some minor modification.

*Experimental animals*

Adult male Wister rats (n=36) weighing (200±20 g) were used for assessment of antidiabetic activity of HGP. The rats were obtained from the animal house of National Research Center (Dokki, Giza, Egypt). The rats were kept under controlled laboratory condition for at least 1 week before starting the experiment at the animal house of Faculty of Veterinary Medicine, Mansoura University. Animals were allowed free access of water and fed a standard rat diet. Animal procedures were carried out in accordance with the guidelines given by the Ethics Committee of the Faculty of Pharmacy (Approval No.: MP1449), Cairo, Egypt, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

*Induction of experimental diabetes*

Type II diabetes was induced in overnight-fasted rats by a single intraperitoneal (*i.p.*) injection of

freshly prepared STZ (40 mg/kg body wt., dissolved in 0.1 M citrate buffer, pH 4.5). After five days, only rats with blood glucose level >250 mg/dL were considered diabetic and used in this experiment. Normal control rats were injected (*i.p.*) with citrate buffer alone.

*Experimental design*

A total of 36 rats were randomly divided into six groups of 6 each as follows: Gr. I, normal control group that received distilled water; Gr.II, diabetic untreated control and received distilled water; Gr. III, diabetic and received HGP at dose 100 mg/kg; Gr. IV, diabetic and received HGP at dose of 200 mg/kg; and Gr. V, diabetic and received metformin at dose of 150 mg/kg. Animals administrated the oral treatments using gastric gavage once daily for 4 weeks. Pectin and Metformin were suspended in water.

*Measurement of blood glucose, insulin and biochemical parameters*

Blood samples were obtained from the tail veins of rats after fasting overnight. Blood glucose was measured with an Accu-check Advantage glucometer and blood glucose test strips (Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin levels were determined by using an insulin ELISA kit with rat insulin as a standard (ALPCO Diagnostics, Windham, NH) according to the manufacturer's instructions. Malondialdehyde (MDA) as oxidative stress marker was determined spectrophotometrically according to Buege & Aust[22]. Serum levels of cholesterol, triglyceride, creatinine, urea, aspartate aminotransferase (AST) (MAK055) and alanine aminotransferase (ALT) (MET-5124), alkaline phosphatase (ALP) (ab83369), albumin (MET-5017) and globulin (500-0006) were determined using commercial diagnostic kits purchased from Biodiagnostic for diagnostic reagents (Dokki, Giza, Egypt).

**Statistical analysis**

Data are expressed as means ± SD. Statistical analysis are done using one way analysis of variance (ANOVA) using (SPSS) software version (10) (Chicago, IL, USA) with least significance level (LSD) between groups at $P < 0.05$.

## Results and Discussion

### Chemical analysis

*Analysis of essential oils*

The highest yield of essential oil from the orange peel was obtained by cold press method of the fresh peel (EO-1, 0.72% w/w), followed by that prepared from the dried peel using hydro-distillation for 30 min (EO-3, 0.6% w/w) and finally, the oil prepared from fresh peel by hydro-distillation for 5 h (EO-2, 0.2% w/w). Analysis of the GC–MS spectra of the three essential oils Suppl. Table 1S. *All supplementary data are available only online along with the respective paper at NOPR repository at http://nopr.res.in*) led to the identification of 29 compounds representing (99.95%), (99.67%) and (99.62%) of the total oil in EO-1, EO-2 and EO-3, respectively. Number of essential oil components obtained from fresh and dried peels by hydro-distillation was higher than that obtained from fresh peel by cold press. Limonene was the major component in all the 3 oil samples (95.07, 90.11 and 94.67%, respectively) in agreement with reported data[23], followed by β-myrcene, α-pinene and linalyl anthranilate. Similar results were previously reported on Ethiopian cultivar by Mitiku *et al.*[24] and on blood oranges by Murthy *et al*.[25] .

***Analysis of pectin***

*Extraction yield, color and pH of pectin*

The yield of pectin obtained from orange peel using the conventional method was 19% w/w, while that of the high grade pectin (HGP) after purification using Diaion HP20 was 20.9% (Table S2). Both samples are light brown in color and had pH values of 3.0 and 3.4, respectively.

*Equivalent weight*

Supplement Table S2 shows that equivalent weights of conventional pectin and HGP were 537.63 and 324.675, respectively. According to Chandel *et al*.[26], the equivalent weight might be also dependent upon the amount of free acid. Besides, HGP pectin could be used to produce low sugar or sugarless jellies, as previously reported by Sharma *et al*.[27].

*Degree of esterification* (*DE*)

Based on DE, pectin can be classified as low methoxyl pectin with ≤50% DE and high methoxyl pectin with >50% DE. In this study, DE of conventional pectin and HGP were 39.21 and 32.46, respectively (Table S2). Therefore, our samples were classified as low methoxyl pectin and this attributed to the use of mature fruit peel, as previously reported by Sundar Raj *et al*.[28].

*Methoxyl content*

Methoxyl content is an important parameter to control the setting time of pectin and the ability of the pectin to form gels[29]. In this study, methoxyl content of conventional pectin and HGP was 3.71 and 4.59,

respectively (Table S2). Based on methoxyl content values, the prepared pectin of all samples was categorized as low methoxyl pectin. The methoxyl content of commercial pectin usually varies from 0.2-12% depending on the source and mode of extraction[30], thus, the values falling within range. Since all the values obtained experimentally were below 7%, hence the pectin were of low ester value indicating that the pectin is good in terms of quality.

*Total Anhydrouronic acid content* (*AUA*)

According to Food Chemical Codex[31]; the AUA indicates the purity of the extracted pectin and its value should not be less than <65%. In this study AUA of conventional pectin and HGP was 53.86% and 80.26%, respectively (Table 2S). These findings revealed that Diaion HP20 is an effective packing material for chromatographic purification of pectin.

*FT-IR analysis*

The FT-IR spectrum of the HGP showed characteristics absorption bands identical to that of standard pectin at 3421.72 $cm^{-1}$ (for O-H stretching vibration), the 2924.09 $cm^{-1}$ (for C-H stretching of the $CH_2$ group), and two bands at 1620.1 and 1446.6 $cm^{-1}$ which can be ascribed to vibrations of the O-C-O structure. The band appears at about 1747.5 $cm^{-1}$ can be assigned to the C-O stretching vibration of methyl esterified carboxylic group. The absence of twin peaks at 3500-3300 $cm^{-1}$ (-$NH_2$) indicated the absence of protein. Moreover, the fingerprint region in FT-IR spectrum of HGP is approximately identical to that of standard pectin (Sigma-Aldrich chemicals, Germany) (Suppl. Fig. 1S A and B).

**Biological assessment**

*Antimicrobial activity of essential oil*

The minimum inhibitory concentration **(**MIC) against all tested micro-organisms was determined. The samples EO-2 (from fresh peel by hydro-distillation for 5 h) showed significant antimicrobial activity against most tested bacterial and fungal strains, with the lowest MIC values (1.95, 1.95, 0.98, 0.98, 0.98 and 1.95 µg/mL) against *E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus*, *E. faecalis* and *A. flavus*, respectively. EO-1 (from fresh peel by cold press) was active only against *S. typhi* and *E. faecalis* with MIC value (0.98 and 1.95 µg/mL), respectively. EO-3 (from dried peels by hydro-distillation for 30 min) showed activity *against C. albicans* and *E. faecalis* as shown in with MIC value (1.95 and 3.9 µg/mL), respectively Tables (3S and 4S).

*Effect of HGP on blood glucose, insulin and biochemical parameters*

In the current study, the effect of HGP on FBG and insulin was assessed in STZ-induced diabetic rats. STZ was used to induce diabetes in the experimental animals, an antibiotic produced by *Streptomyces achromogenes*, which has been intensively used to produce chronic hyperglycemia through its partial damage on pancreatic β-cells. This effect was produced most probably via DNA damage[32], and generation of ROS (from the action of STZ on the mitochondria and from increased xanthine oxidase activity)[33]. In the present study, we observed a significant decrease in insulin levels with concomitant increase in glucose levels of diabetic rats. In the first week; Gr. III & IV (HGP 100 and 200 mg/kg, respectively) showed significant decline in blood glucose level. Moreover, Gr. IV showed significant decrease in blood glucose level more than Gr. V (metformin treated group) by 25.31% as shown in Fig. 1 and Suppl. Table S5. In the second week; Gr.III that received HGP (100 mg/kg) showed significant difference in comparison with Gr. V (metformin treated group) and there was no difference between Gr. IV and V as shown in Fig. 1. In the third week; Gr. IV showed better reduction than Gr. V (metformin treated group). At the end of the fourth week; Gr. III & IV showed close values to Gr. V (metformin group) as shown in Fig. 1 and Suppl. Table S5. Also, administration of HGP (200 mg/kg) was effective to not only achieve rapid and significant decline in BGL in comparison with Gr. V (metformin treated group)

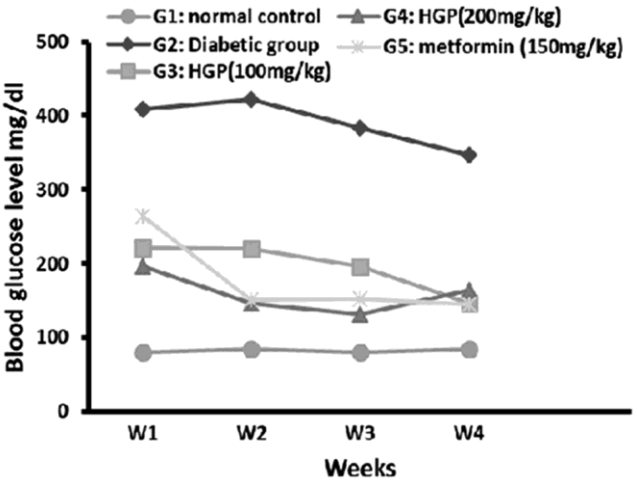


Fig. 1 — Effect of HGP on BGL of STZ induced diabetic rats during 4 weeks. [HGP, high grade pectin; Statistical analysis were done using one way analysis of variance (ANOVA) gram with least significance level (LSD) between groups at $P$ <0.05]

for the first week, but also had the ability to show gradual and significant decrease in the BGL during the four weeks. Finally, groups III, IV & V demonstrated significant decline in blood glucose level all over the four weeks of treatment by 57.9%,, 52.7%, and 58.3%, respectively (Fig. 1 & Suppl. Table S5). These findings seem to contradict with another study by Liu *et al*.[34] who reported that there was no significant difference in FBG level between the diabetic control and pectin groups at the start of the study ($P$ >0.05); however, FBG levels in the diabetic control group and citrus pectin groups (500, 1000 and 2000 mg/kg) were higher than those in the normal control group. Also, the authors reported that after two weeks of citrus pectin administration, the medium- and high- dose groups showed significantly lower FBG levels compared with those in the diabetic control group. After four weeks of treatment, significantly decreased FBG levels were observed in all three citrus pectin groups and the metformin-treated group compared with those of the diabetic control group ($P$ <0.05 or $P$ <0.01). Moreover, the FBG levels in the three citrus pectin groups were significantly higher than those in the metformin group[26]. This may be due to the purification process using Diaion HP20 or due to the nature of the molecular structure of the HGP isolated from Egyptian *citrus*.

After four weeks, the oral administration of HGP (200 mg/kg) caused significant increase in serum insulin level (26.56 mIU/L) when compared to normal control (2.72 mIU/L) as shown in Fig. 2A. This observation suggests that pectin positively enhanced insulin secretion, which supports, at least in part, its effect as insulinomimetic agent; in agreement with data previously reported by Liu *et al*.[34]. In addition, HGP showed improvement in insulin resistance factor as shown by increase of HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) in both doses (Suppl. Table S5).

*Effect of HGP on oxidative stress marker*

The effect of HGP on the level of MDA; a product of lipid peroxidation, was determined in STZ-induced diabetic rats after four weeks. In untreated diabetic rats, MDA level was significantly increased by 43%. However, rats received HGP showed significant reduction in MDA level, which was almost similar to that of normal rats as shown in Fig. 2B. HGP showed powerful antioxidant activity to reduce lipid peroxidation in STZ-induced diabetic rats.

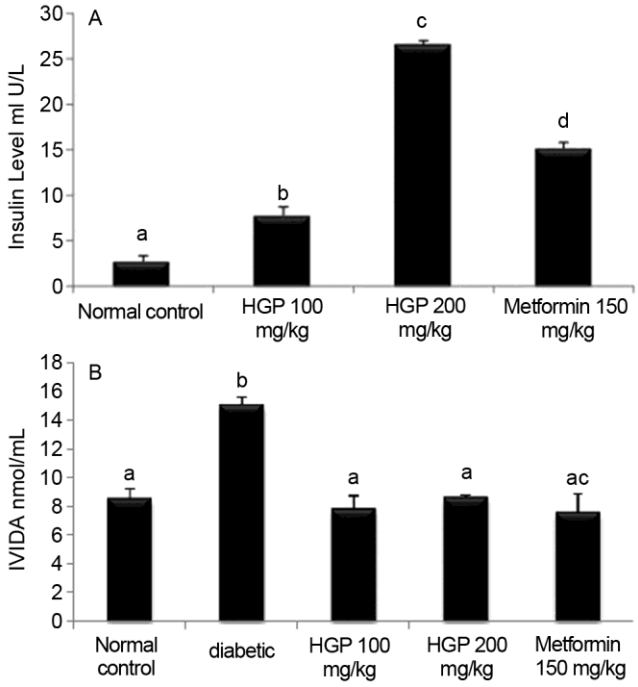


Fig. 2 — Effect of HGP on (A) insulin; and (B) MDA level after 4 weeks of treatment. [HGP, high grade pectin; Statistical analysis was done using one way analysis of variance (ANOVA) with least significance level (LSD) between groups at $P$ <0.05. Different superscript letters (a, b, c, and d) indicate significant difference]

*Effect of HGP on serum biochemical parameters of liver and kidney*

Liver function parameters (AST, ALT and albumin) were improved by administration of HGP (100 and 200 mg/kg) when compared to diabetic untreated control. Moreover, AST and ALT showed no significant difference when compared to control. The value of Albumin/Globulin (A/G) showed improvement by administration of HGP (100 and 200 mg/kg) when compared to diabetic rats as shown in Fig. 3 A-E. The total protein and ALP values of both liver and kidney were improved by administration of HGP (200 mg/kg) as shown in Fig. 4 A and B. Creatinine and BUN were also improved by administration of HGP (100 mg/kg and 200 mg/kg) when compared to diabetic rats as shown in Fig. 5 A and B. This finding is in agreement with data reported by Khotimchenko *et al*.[35], who found that low methoxyl pectin lowered blood urine and creatinine levels, and increased daily diuresis in rats with experimental renal failure. Also, Shtriker[36] reported that pectin slowed the progression of renal failure but also improved serum levels of total protein, albumin, globulin, albumin/globulin (A/G), serum Na & K and liver enzymes.

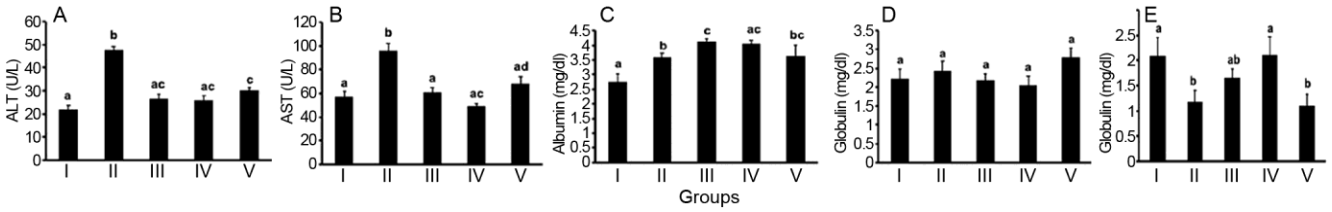


Fig. 3 — Effect of HGP on different liver biomarkers: (A) ALT; (B) AST; (C) Albulin; (D) Globulin; and (E) A/G. [Groups: I, Normal Control; II, Diabetic; III & IV, HGP@100 & 200 mg/kg; and V, Metformin @150 mg/kg. HGP, High grade pectin; Data are mean ±SD of six rats in each group. Statistical analysis is done using one way analysis of variance (ANOVA) using SPSS software version (10) with least significance level (LSD) between groups at $P$ <0.05. Different superscript letters (a, b, c and d) means; there is significant difference]

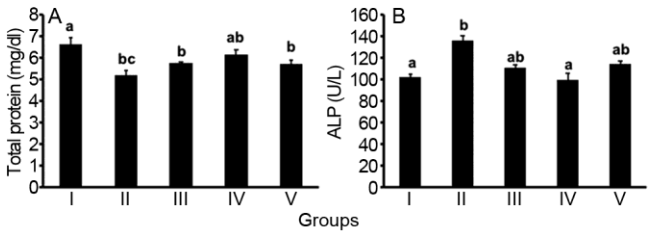


Fig. 4 — Effect of HGP on (A) total protein; and (B) ALP. [HGP, High grade pectin. Data are mean ±SD of six rats in each group. Statistical analysis is done using one way analysis of variance (ANOVA) with least significance level (LSD) between groups at $P$ <0.05. Different Superscript letters (a, b, c and d) means; there is significant difference]

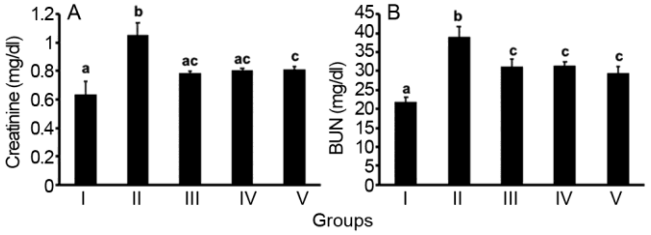


Fig. 5 — Effect of HGP on different kidney biomarkers. [HGP, High grade pectin. Data are mean ±SD of six rats in each group. Statistical analysis are done using one way analysis of variance (ANOVA) with least significance level (LSD) between groups at $P$ <0.05. Different Superscript letters (a, b, c and d) means; there is significant difference]

The levels of cholesterol (TC) triglycerides (TG) were improved by administration of both doses of HGP when compared to diabetic rats (Fig. 6 A & B). Moreover, the higher dose of HGP showed significant reduction in the levels of TC and TG comparable to that of metformin treated group. These results were in agreement with Liu *et al.*[34] who reported that citrus pectin treatments exhibited dose-dependent decreases in the levels of TG and TC. Khotimchenko *et al.*[35] reported that pectin with low esterification lowered creatinine level and increased daily diuresis in rats with experimental renal failure.

Accordingly, we suggest that pectin with chemical structures having low degree of esterification (<50%) and high percentage of AUA, such as HGP (32.46 and

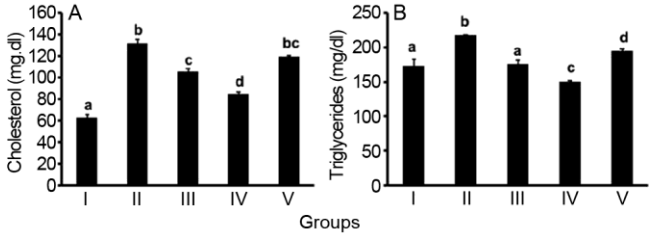


Fig. 6 — Effect of HGP on (A) cholesterol; and (B) triglycerides. [HGP: High grade pectin. Data are mean ±SD of six rats in each group. Statistical analysis is done using one way analysis of variance (ANOVA) with least significance level (LSD) between groups at $P$ <0.05. Different Superscript letters (a, b, c and d) means; there is significant difference]

80.26%, respectively) could be considered as potential agent for the management of diabetes and its metabolic disorders. However, further research is required to clarify the mechanism of its antidiabetic effect.

## Conclusion

In the present study, high-value materials; essential oil (EO) and high grade pectin (HGP) were prepared from orange processing waste (peels). Cold press method has been observed to be the most applicable method to isolate orange oil on pilot scale which gave the highest yield (0.72% w/w), and the EO obtained showed potent antimicrobial activity against most food borne pathogens. Therefore, it could be utilized as one of the important food preservatives. On the other hand, a high value and good quality pectin; HGP was obtained from the waste of orange juice processing industry from the crude acidic water extract of the peel after passing through Diaion HP20 column. It is worth to mention that the HGP has low DE (Degree of esterification) and methoxyl content, and high percentage of anhydrouronic acid. On the other hand, the high percentage of anhydrouronic acid (AUA) content reflects purity of HGP and indicates that Diaion HP20 resin is an effective packing material for pectin purification. The HGP prepared demonstrated potent antidiabetic activity at lower

dose than those reported in the literature. It possessed significant ability to improve blood glucose level, as well as parameters relevant to liver and kidney functions in STZ-induced diabetic rats. Accordingly, we suggest that pectin with chemical structures having low DE (<50%) and high percentage of AUA, could be considered as potential agent for the management of diabetes and its metabolic disorders.

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**Conflicts of interest**

Authors declare no competing interests.

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