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# Estimation of resveratrol content in peanut pericarp and its relation to the *in vitro* inhibitory activity on carbohydrate metabolizing enzymes

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The aim of this work was the estimation of resveratrol content in two successive extracts (EtOAc and MeOH) of peanuts (*Arachis hypogaea* L.) pericarp of Egypt, by TLC and HPLC methods. Results showed the presence of 3.0 and 0.5 μg/mL resveratrol in EtOAc and MeOH extracts respectively. The *in vitro* carbohydrate hydrolyzing enzyme inhibition activity showed higher percentage of inhibition of α–amylase, α–glucosidase and β-galactosidase with EtOAc (4.32, 5.93 and 13.7%) than with MeOH extract (3.9, 4.9 and 14.1%) but lower than the standard resveratrol concentration (5.18, 5.94 and 13.26%) and the reference acarbose (5.88, 5.9 and 13.0%). It could be concluded that the content of resveratrol in peanut pericarp is related to the percentage of inhibition activity of carbohydrate hydrolyzing enzymes. These results strongly reflect the benefit of using peanut pericarp, the waste product, as a natural antidiabetic agent.

## 1. Introduction

The peanut (*Arachis hypogaea* L.), is a dehiscent legume, harvested from below the soil and grown primarily for use as food either as shelled nuts or as a source of the seed oil, but peanut shells (hulls, seed coats), produced in hundreds of thousands of tons annually as by- products of the peanut industry, still do not have any significant use or value (Sobolev and Cole 2003). The prominent antioxidant activity of the methanolic extracts of peanuts hulls was previously reported (Lee et al. 2006). Diabetes mellitus is a complex metabolic disease. The Middle East and Northern Africa (MENA) have the highest prevalence of diabetes as a world region. Egypt is number nine among the top 10 ranking (MENA) countries. More than 11% of the population of Egypt suffers from type 2 diabetes (http://www.diabetes24-7.com/?p = 611). The hypoglycemic and hypolipidemic effects of an aqueous extract of *A. hypogaea* seeds were reported, while the aqueous extract caused a significant decrease in total cholesterol, serum triglycerides, HDL and LDL-cholesterol in both normal and alloxan-induced diabetic rats (Moreno et al. 2006). Diabetes and obesity are generally linked to complications in lipid metabolism and oxidative stress. Inhibition of the digestion and absorption of dietary fat and carbohydrates have been used as target in obesity and diabetic treatment (Moreno et al. 2003). α-Amylase, α-glucosidase, β-galactosidase and pancreatic lipase are important enzymes for the digestion of dietary carbohydrate and triacylglycerols. *Trans*-resveratrol (*trans*-3,5,4'-trihydroxystilbene), a polyphenolic compound uniquely identified in plants including peanuts (Ragab et al. 2006) greatly contributes to human health. It is a phytoalexin, a class of antibiotic compounds and the major active stilbene that confers pathogen resistance. It can be converted to *cis*-resveratrol when exposed to UV light. Data from the literature indicated the effect of resveratrol in treatment of type 2 diabetes by three main aspects: reduction of blood glucose, improvement in insulin action and preservation of β–cells (Szkudelski and Szkudelska 2011). The present study was undertaken to estimate the resveratrol content in peanut pericarp to elucidate the hypothesis that the presence of the bioactive resveratrol compound in *A. hypogaea* pericarp extract, that may have anti-diabetic activity through the inhibition of carbohydrate metabolizing enzymes *in vitro* and to assess the potential investment of peanuts industry byproducts (peanuts pericarp) for treatment of diabetes.

## 2. Investigations, results and discussion

The total 70% MeOH extract yield 27% of the powdered pericarp. The successive extracts yield 19%, 28% and 27.5% for *n*-hexane, EtOAC and MeOH respectively. TLC screening for resveratrol content showed a bluish violet fluorescent spot at Rf = 0.81 and 0.7 in $S_1$ and $S_2$ respectively in two extracts (EtOAC and MeOH), which did not change with $NH_3$ vapors, beside other components. $^1$H NMR spectrum of the total 70% MeOH extract (Fig. 1) showed peaks between 6–7.3 ppm, indicating the presence of phenolic contents, between 4 and 5 ppm, indicating the sugar content as well as the peaks at 6.26 (triplet) and 7.37 (doublet) of resveratrol (3,5,4'-trihydroxystilbene) (Mannila et al. 1993). For HPLC analysis, results of method validation and limit of detection (LOD) and limit of quantitation (LOQ) are presented in Table 1. Good recovery and accuracy of the method is apparent from the results listed in Table 2. No chromatographic changes were observed when resveratrol working solution was stored for 3 days at room temperature and protected from light, so the solution can be regarded as stable. RP-HPLC technique was chosen for analysis of the marker compound resveratrol in the two extracts and the result is presented

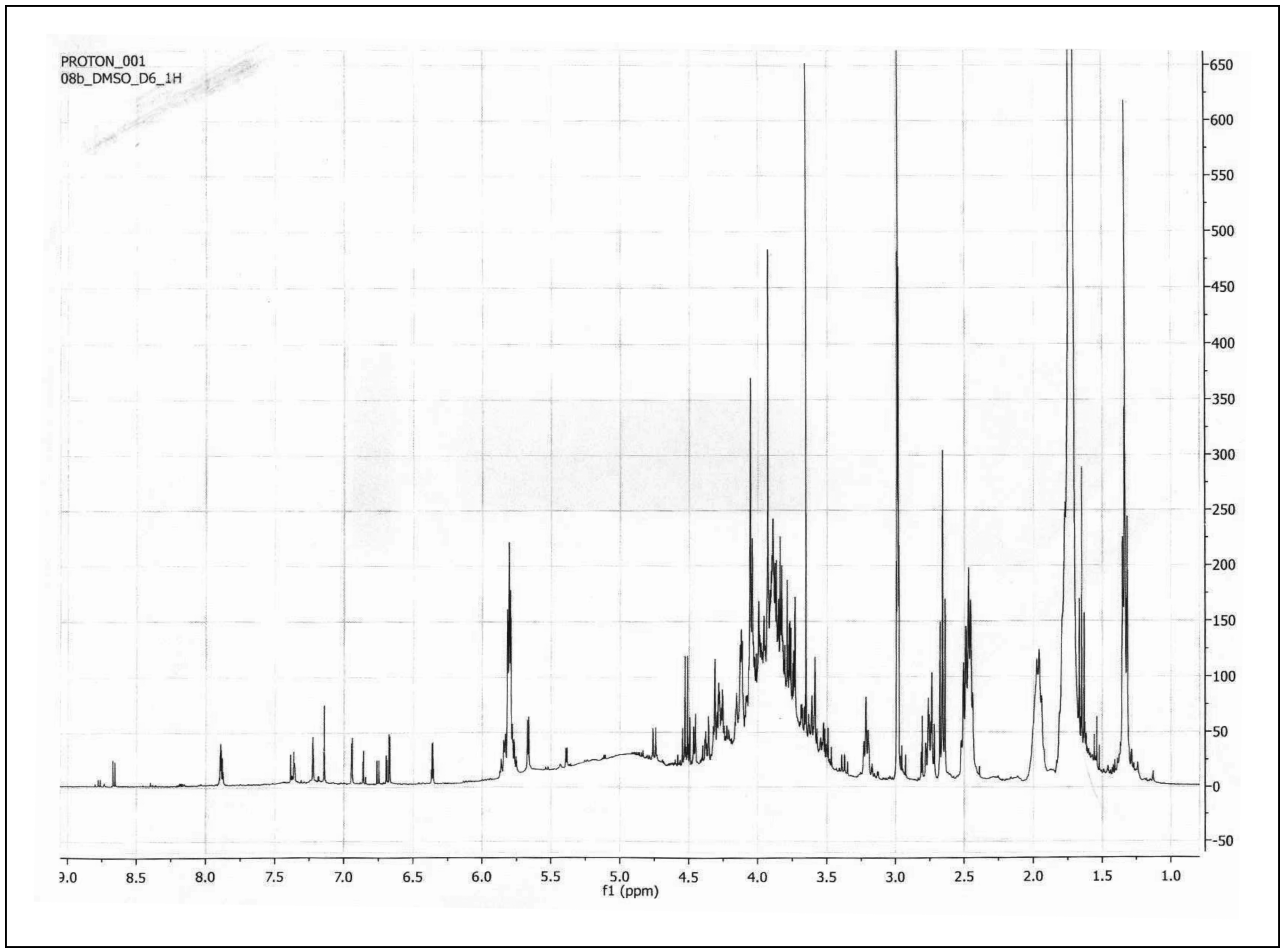


Fig. 1: $^{1}$H NMR spectrum of total 70% methanol extract of peanuts pericarp.

in Fig. 2. The concentration of resveratrol, which appeared at $t_{R=}$ 8.0 min (Fig. 2a), was higher in EtOAc: 3 mg/ml (Fig. 2 b) than MeOH: 0.5 mg/ml (Fig. 2c) extract.

All the tested extracts showed appreciable carbohydrate inhibitory activities. Resveratrol was the most active (5.18%, 5.94% and 13.26%) followed by EtOAc extract (4.32%, 5.93% and 13.7%) in inhibition of α-amylase, α-glucosidase and β-galactosidase respectively when compared to the standard acarbose (5.88%, 5.9% and 13% respectively). However MeOH extract showed the lowest inhibitory activity (3.9%, 4.9% and 14.1% respectively). A dose–response relationship was found for the carbohydrate inhibitory effect. The activity increased as the concentration of the tested extract increased in each case (Table 3). We can deduce a significant increase in reducing activity with the increase in concentrations of the extracts (linear relationship) and at low doses the reducing activity shows insignificant change. One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes: α-amylase, α-glucosidase and β-galactosidase in the digestive tract. Inhibition of these enzymes delays carbohydrate digestion and prolongs overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Rhabasa–Lhoret and Chiasson 2004).

The use of complementary or alternative products for human health is increasing worldwide. Over the past two decades, numerous health benefits impacting cardiovascular disease, various cancers, atherosclerosis and aging have been linked with resveratrol (Baur and Sinclair 2006). Resveratrol is a well known and well studied phytochemical, several other resveratrol derivatives have also been shown to have similar and/or additional health benefits.

The anti-hyperglycemic effect of resveratrol could be due to its stimulatory action on intracellular glucose transport by causing an increase in glucose uptake by different cells in absence of insulin. It is also able to attenuate cytokine-induced toxicity which reduces the oxidative damage of the pancreas, and

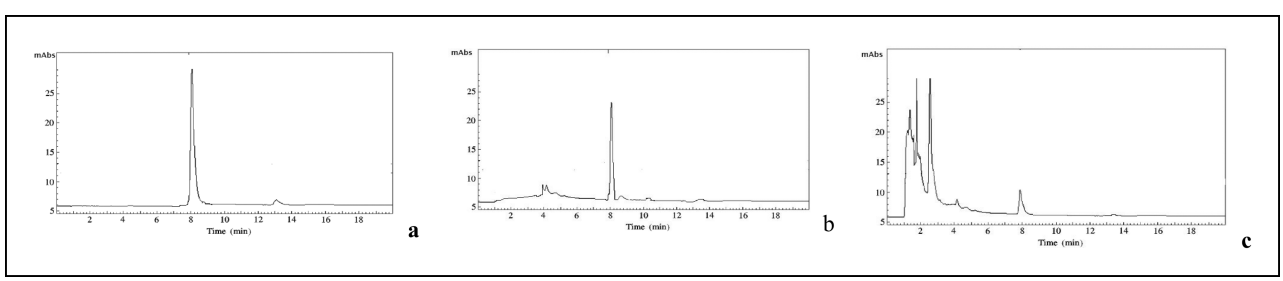


Fig. 2: HPLC chromatograms of (a) resveratrol standard; (b) EtOAc extract and (c) MeOH extract of peanut pericarp.

**Table 1: Method validation data obtained by use of the proposed RP-HPLC method for analysis of pure resveratrol**

| | |
|---|---|
| Linear range (μg/mL) | 5–50 |
| LOD, (μg/ mL) | 3.85 |
| LOQ, (μg/ mL) | 4.7 |
| Slope | 0.7321 |
| Intercept | 0.4768 |
| Correlation coefficient | 0.9982 |
| Mean | 99.34 |
| SD | 1.34 |
| % RSD[a] | 1.61, 0.77, 0.74 |
| % RSD[b] | 1.48, 1.45, 1,33 |

LOD: Limit of detection; LOQ: Limit of quantitation; [a]intra-day % RSD for concentrations 10, 30, 45 μg/mL; [b]inter-day % RSD for concentrations 10, 30, 45 μg/mL.

**Table 2: Results from application of the standard addition technique to analysis of resveratrol in ethyl acetate extract**

| Amount of resveratrol (μg/mL) | | Recovery % |
|---|---|---|
| Added | Found | |
| 10 | 10.22 | 102.20 |
| 20 | 19.98 | 99.90 |
| 30 | 30.12 | 100.40 |
| Mean ± S.D. | 100.83 ± 1.21 | |

may ameliorate the endocrine function of this gland (Szkudelski and Szkudelska 2011). Also, stimulation of SIRT1 (an enzyme which regulates the metabolic rate) enzyme activity decreases glucose levels and improves insulin sensitivity (Elliott and Jirousek 2008). SRT-501, the first in a novel class of SIRT1 activators, is a proprietary formulation of resveratrol with improved bioavailability. Presence of resveratrol in a higher amount in the EtOAc extract of our study could explain its more effective antidiabetic action when compared to the MeOH extract. The pure standard resveratrol showed the best decrease in the blood glucose in animals with hyperglycemia when compared to the extracts. These results direct towards the thinking that the antidiabetic effect is mainly due to the content of resveratrol.

## 3. Experimental

### *3.1. Phytochemical study*

#### *3.1.1. Plant material*

Fruits of *Arachis hypogaea* L. (peanut, Fig. 3), were collected from El-Behera Gogernorate in 2010. The plant was kindly identified by Mrs. Therese Labib, Senior Botanist in El-Orman Garden, Egypt. A voucher sample no. BSP25 is desposited in Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Egypt. The fruits were unfolded and the seeds were manually separated. The pericarp (hulls) were air-dried in the shade, powdered and stored in glass containers.

#### *3.1.2. Preparation of the extracts*

The powdered peanut pericarp (100 g) was extracted by maceration, on cold, in a dark container, with 70% MeOH till exhaustion. Combined extracts were filtered and concentrated under reduced pressure. The residue left was shaked with $H_2O$ and successively fractionated with polarity graded solvents, using a separating funnel, starting with *n*-hexane, EtOAC and MeOH. Each of the successive extracts was concentrated and the residue left was weighed, kept in an amber glass container, stored in a refrigerator until used for the phytochemical and biological studies.

**Table 3: Carbohydrates metabolizing enzymes inhibition % of EtOAc and MeOH extracts of peanut pericarp compared to resveratrol standard**

| Concentrations | Acarbose | EtOAc extract | MeOH extract | Resveratrol |
|---|---|---|---|---|
| | | **α-Amylase inhibition %** | | |
| 10 μg/mL | $32.21 \pm 1.23^{a}$ | $17.59 \pm 0.60^{e}$ | $16.48 \pm 0.98^{e}$ | $28.20 \pm 1.97^{d}$ |
| 50 μg/mL | $35.13 \pm 2.44^{c}$ | $28.70 \pm 1.04^{a}$ | $24.78 \pm 2.24^{b}$ | $34.10 \pm 3.03^{c}$ |
| 100 μg/mL | $47.36 \pm 3.10^{g}$ | $34.29 \pm 3.11^{d}$ | $28.34 \pm 1.89^{f}$ | $45.16 \pm 2.12^{g}$ |
| 500 μg/mL | $52.56 \pm 1.69^{c}$ | $45.12 \pm 3.33^{a}$ | $39.65 \pm 2.80^{b}$ | $52.23 \pm 2.29^{c}$ |
| 1000 μg/mL | $71.34 \pm 2.67^{d}$ | $58.10 \pm 2.14^{a}$ | $50.50 \pm 1.10^{b}$ | $67.97 \pm 3.86^{c}$ |
| LSD 5% | 5.88 | 4.32 | 3.9 | 5.18 |
| | | **α-Glucosidase inhibition %** | | |
| 10 μg/mL | $29.95 \pm 2.00^{b}$ | $25.15 \pm 3.00^{c}$ | $23.00 \pm 3.06^{c}$ | $28.95 \pm 2.63^{b}$ |
| 50 μg/mL | $43.23 \pm 3.08^{c}$ | $34.81 \pm 23.16^{b}$ | $33.99 \pm 2.70^{b}$ | $37.90 \pm 2.15^{a}$ |
| 100 μg/mL | $52 .33 \pm 4.07^{d}$ | $45.20 \pm 2.39^{d}$ | $41.00 \pm 2.00^{a}$ | $46.21 \pm 2.87^{c}$ |
| 500 μg/mL | $69.12 \pm 3.10^{c}$ | $48.00 \pm 2.00^{d}$ | $42.61 \pm 1.79^{a}$ | $55.79 \pm 1.50^{b}$ |
| 1000 μg/mL | $85.89 \pm 2.28^{c}$ | $64.39 \pm 1.90^{d}$ | $59.91 \pm 2.90^{a}$ | $69.70 \pm 2.28^{b}$ |
| LSD 5% | 5.90 | 5.93 | 4.90 | 5. 94 |
| | | **β-Galactosidase inhibition %** | | |
| 10 μg/mL | $45.85 \pm 2.90^{a}$ | $23.12 \pm 5.31^{b}$ | $17.90 \pm 4.32^{c}$ | $34.35 \pm 3.20^{d}$ |
| 50 μg/mL | $55.90 \pm 4.70^{a}$ | $34.78 \pm 4.90^{b}$ | $35.35 \pm 9.00^{b}$ | $39.62 \pm 8.90^{c}$ |
| 100 μg/mL | $59.00 \pm 6.10^{a}$ | $39.59 \pm 8.35^{b}$ | $36.80 \pm 9.38^{c}$ | $53.49 \pm 8.80^{d}$ |
| 500 μg/mL | $78.56 \pm 5.78^{a}$ | $62.23 \pm 2.99^{b}$ | $54.70 \pm 4.41^{c}$ | $69.78 \pm 4.90^{d}$ |
| 1000 μg/mL | $83.66 \pm 6.51^{a}$ | $65.10 \pm 5.07^{b}$ | $56.00 \pm 5.01^{c}$ | $72.86 \pm 5.01^{d}$ |
| LSD 5% | 13.00 | 13.7 | 14.1 | 13.26 |

α-amylase, α -glucosidase and β –galactosidase are expressed as %; Data are mean ± SD of triplicates; Statistical analysis with one way analysis of variance (ANOVA) using CoStat computer program; Unshared superscript letters between treatments are significance values at $P < 0.05$

Fig. 3: Pericarp of peanuts.

*3.1.3. Qualitative analysis of resveratrol by TLC and $^1$H NMR*

Aliquots of each of the successive extracts and the pure authentic *trans*–resveratrol (resveratrol) (Sigma, USA, purity 99.8%) were spotted on precoated silica gel 60 $F_{254}$ plates for TLC (E. Merck, Darmstadt, Germany) using two different solvent systems $S_1$: EtOAc/AcOH/$H_2O$ (17:1:2) and $S_2$: hexane/EtOAc (1:1) and the plates were visualized under UV for checking the presence of resveratrol. The total 70% MeOH extract was analysed by $^1$H NMR spectral technique (Varian Unity INOVA, 400 MHz spectrometer) and the result is presented in Fig. 1.

*3.1.4. Quantitative analysis of resveratrol by HPLC*

3.1.4.1. Chemicals. Methanol and acetonitrile for HPLC (Sigma Aldrich, Germany), orthophosphric acid (El–Nasr Pharmaceutical Chemicals Co., Egypt) and deionized water (SEDICO pharmaceutical Co., Egypt) were all of analytical grade and were used without further purification.

3.1.4.2. Preparation of standard solutions and calibration. A standard stock solution of resveratrol (1 mg/mL) was prepared and stored protected from light at 4 °C until used. Working standard solution of resveratrol (100 μg/mL) was prepared by appropriate dilution of the standard resveratrol stock standard solution. Calibration graphs for HPLC were recorded with sample amounts ranging from 5–50 μg/mL and prepared by appropriate dilution of resveratrol. The solutions were then analysed and their peak areas were recorded. Validation was performed according to ICH guidelines (ICH 2005). The regression equation was calculated as follows: $Y = 0.7321C \times -0.4768$; $r = 0.9982$, where Y is the peak area x $10^{-4}$, C is resveratrol concentration in μg/mL respectively and r is the correlation coefficient.

3.1.4.3. HPLC analysis conditions. All separations were performed on a Shimadzu Class-LC 10 AD Liquid Chromatograph equipped with a Shimadzu SPD-10 A UV-VIS Detector (Shimadzu Corporation, Japan), vacuum degasser: Shimadzu degasser (DGU-3A). A Phenomenex C18 (25 cm × 4.6 mm i.d, 5 μm particle size) column was used as a stationary phase for HPLC determinations (USA). Isocratic elution was carried out using acetonitrile/water (25:75, v/v) pH = 3.5 with orthophosphoric acid as a mobile phase at a flow rate 1.5 mL/min at room temperature, $t_R$ (retention time) = 5.8 min. The injection volume was 20 μL and the analytes were detected at 310 nm. Quantitative determination was carried out by the external standard method based on peak highs.

***3.2. Biological study***

*3.2.1. Chemicals*

All chemicals were of analytical grade: Sigma, Merck and Aldrich. All kits: Biosystems (Spain), Sigma (USA) and Biodiagnostic (Egypt). Purified enzymes; carbohydrate metabolizing enzymes; α-amylase, α-glucosidase and β-galactosidase (EC3.2.1.1, EC3.2.1.20 and EC3.2.1.23 respectively): Sigma (USA).

*3.2.2. Carbohydrates hydrolyzing enzymes inhibition assay*

*α-Amylase inhibitory activity* was determined according to the method described by Ali et al. (2006) by measuring the absorbance at 540 nm.
The inhibitory activity of β-galactosidase was measured by the method of Sanchez and Hardisson (1980) and the resulting color of O-nitrophenolate ions was measured at 420 nm. A standard curve was performed using different concentrations of O-nitrophenol.
α-Glucosidase inhibitory activity was determined according to the method of Kim et al. (2005) and the reducing activity was estimated by measuring release of *p*-nitrophenol from *p*-nitrophenyl α-D-glucopyranoside at 405 nm. A standard curve was carried out using different concentrations of *p*-nitrophenol.

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