Egyptian Plantagoovata Seeds: Nutritional, Biological Activities, and Chemical Constituents

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Abstract

This study sought to describe the chemical composition of the wild Plantagoovata in order to ascertain its antioxidant and anticancer properties. An ethanol extract of Plantagoovata seeds was tested against three different human cell lines, including colon adenocarcinoma (HCT-116), hepatocellular carcinoma (HepG-2), and human breast cancer (MCF-7) cell lines, after its chemical components were described and identified by GC-MS. Using the DPPH radical scavenging technique, the antioxidant activity of Plantago ovata's ethanol extract was evaluated. The capacity of each antidiabetic agent to inhibit human α-amylase and α-glucosidaseswas then assessed, and results were compared to those of the antidiabetic medication acarbose. Additionally, it was predicted that inhibiting pancreatic lipase might clarify Plantago's

effects.17 compounds from an ethanol extract of Plantago ovata were characterized and recognized by GC-MS for the first time. When compared to vitamin C, the antioxidant activity of Plantago ovata's ethanol extract demonstrated a comparably strong antioxidant, scavenging affinity against DPPH radicals. Additionally, Plantago ovata's ethanol extract's anti-cancer properties had good antitumor properties against all examined cell lines. The inhibitory actions of Plantago ovata on human -glucosidases and -amylase may be useful in regulating postprandial glycaemia and sugar absorption without the drawbacks of acarbose therapy.

Keywords: Plantagoovata,anticancer activity, antioxidant activity, antidiabetic activity.

Introduction

A key component of non-timber forest products is medicinal herbs. In rural places all around the world, these plants are regarded as a crucial component of healthcare. The majority of medicinal plants are used as a source of raw materials to create traditional and contemporary medicines, nutraceuticals, food supplements, folk remedies, pharmaceutical intermediates, and chemical entities for synthetic pharmaceuticals. Medicinal plants used locally as self-medication for skin conditions and natural cosmetics ***(Nisaret al., 2018)***. Plants are the source of food, feed, energy, and important pharmaceutical substances ***(Mani et al., 2014).*** Due to the presence of phytotoxic secondary metabolites such alkaloids, glycosides, flavonoids, terpenoids, steroids, saponins, tannin, coumarins, and carbohydrates, medicinal plants

have anti-inflammatory, antiviral, anti-cancer, anti-microbial, anti-tumor, and mutagenic properties

(Larayetanet al., 2019& Wink, 2015).

Psyllium, also known as plantago ovata in science, has developed a reputation as a useful natural remedy ***(Akbar, 2020).*** Psyllium is the common name for a number of species in the Plantago genus, and other generic names for this important plant include Psyllium husk and Ispaghula husk. There are more than 200 species in the genus Plantago, which is grown all over the world. Due to its seed mucilage, medicinal, cosmetic, and food grade qualities, P. ovata and P. psyllium are cultivated economically in various American, South Asian, and European countries as a major seasonal crop **(*Zhang et al., 2021).***

The existence of several phytoconstituents such alkaloids, tannins, glycosides, saponins, flavonoids, and phenols was confirmed by phytochemical analysis of the Plantagoovata***(Abbas et al., 2021&Frezza et al., 2022 &Sharmaet al., 2017).***

Metabolites, FAs, amino acids, total phenolics, total flavonoids, antioxidants, and scavenging activities altered gradually and were coordinatedly connected to one another during fruit growth ***(Nuerxiati et al., 2022&Patelet al., 2020).*** Plantago ovata is a well-known medicinal plant in the treatment of inflammatory bowel disease**(*Reddyet al., 2018)***, exhibit cholinergic properties***(Zengin et al., 2019),*** decrease blood pressure***(Tong et al., 2019)***, reducing cholesterol level***(Jovanovski et al., 2018)*** and showed good antibacterial activity***(Nosratabadi et al., 2015).***

Materials and methods:

Collection and preparation of extracts of plant material

The plant was obtained at a local market in Egypt in September 2021, and the botany department of the Faculty of Science at Zagazig University identified it. The voucher specimen for the plant was 100086592. (S). Using 90% ethanol, 0.5 kg was extracted thoroughly over the course of 24 hours. After being filtered using filter paper, the extract was collected and concentrated using a Rota-vapor to produce a solid gum that was then tested by GC (Gas chromatography) ***(Selim & Abd El-Azim, 2020)***.

GC-MS (Gas Chromatography/Mass Spectrometry) analysis: The analytical GC-MS analyses were according to ***(Selim & Sakeran, 2014).***

Material and methods for biological activities

MTT assay

Humancolon carcinoma cells (HCT-116), hepatocarcinoma cell lines (Hep-G2), breast adenocarcinoma cells (MCF-7) was purchased from ATCC, USA, were used to evaluate the cytotoxic effect of the tested extract. Cytotoxicity of tested samples was measured against different tumor cells using the MTT Cell Viability Assay***(Tavallaii et al., 2010).***

Antioxidant activity (DPPH assay)

The free radical scavenging activity was determined according to the method of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical**(*Kilani et al., 2005)***. Different concentrations (0.025-0.4 mg/ml) of Plantago ovata extract (0.5 ml) were mixed with 0.5 ml

freshly prepared DPPH in MeOH. The absorbance was measured at 517 nm after incubation for 30 min in the dark condition.

The Analysis of Underivatized Amino Acids by HPLC

Table 1 lists the parameters for the HPLC method, while Table 2 lists the parameters for the MS method. Filtered via 0.45-m filters, all of the solvents and diluents employed were of HPLC grade. 0.1N HCl was utilised as the diluent. Thermo ScientificTM provided the Pierce 17-Amino Acid Standard H Mix (protein hydrolysate) (Rockford, Illinois). Aspartic acid (Asp), glycine (Gly), glutamic acid (Glu), isoleucine (Ile), histidine (His), lysine (Lys), leucine (Leu), methionine (Met), proline (Pro), phenylalanine (Phe), serine (Ser), tyrosine (Tyr), threonine (Thr), and valine were among the amino acids that were present (Val). Each amino acid was present in 0.1N HCl at a concentration of 2.50 mol/mL ***(Villanueva-Gutiérrez et al., 2022).***For the working standard 1 (WS1) and the working standard 2, the standard was diluted with diluent to 1.0 mol/mL and 0.1 mol/mL, respectively (WS2). Because alanine and cysteine had lesser sensitivity than other amino acids, they were calibrated using WS1. Through serial dilution with diluent, the lower level standards were created from each of the two working standards. All calibrants and samples were filtered through 0.45-m filters to get rid of any tiny particles before injection.

Anti-diabetic assay

Animal selection and induction of diabetes

Thirty-six adult male albino rats, weighting (160±5g), were used in this study. The animals were put in separate cages under 50-60% humidity and were fed on basal diet and water was provided to the rats by means of glass tube projecting through the cage wire. The basal diet was prepared from fine ingredients (100

g) according to ***Reeves et al., (1993).*** Rats were divided in six groups with 6 animals in each group. One group served as a normal control (Group1),the secand group (30 rats) was injected with freshly prepared Alloxan in saline at a dose level of 150 mg/ kg body weight ***(Lazarow&Palay, 1954, Wohaieb& Godin, 1987&Kakkar, et al., 1998).*** After all rats with FBG >126 mg/dl were considered to be diabetics, they were

divided into five groups as follows:

Group 2 was considered as control positive and was fed on basal diet.

Group 3 was fed on basal diet + 5% Seeds of Plantagoovata

Group 4 was fed on basal diet + 10% Seeds of Plantagoovata

Group 5 was fed on basal diet of + 0.5 mm Seeds of Plantagoovata extract orally.

Group 6 was fed on basal diet + 1mm Seeds of Plantagoovata extract orally.

At the end of experiment, blood samples were collected for biochemical analyses

Biochemical analyses

Erythrocytes and serum samples were used for determination of glucose***(Young, 2001)*****,** lipase***(Liu, X et al., 2022)*** , creatinine***(Liu, C et al., 2011)***, uric acid***(Schultz & Moore, 1984),*** urea***(Li et al., 2005),*** α-amylase***(Visvanathan et al., 2016)***, α-Glucosidase***(Bhatia et al., 2019).***One-way ANOVA was used to analyse the data in SPSS; P values under 0.05 and over 0.01 were regarded as statistically significant ***(Alnamiet al., 2022).***

Results

Chemical constituents of the Ethanol extract of Plantagoovata

GC-MS was used to analyse and identify the chemical components of an ethanol extract of Plantagoovata. The results are shown in figure 1 and table 1. Additionally, as can be seen from the results in figure 1 and table 1 above, seventeen components were characterised and identified by using GC-MS of Plantagoovata ethanol extract using real samples.

Anti-tumor activity

The HepG-2, MCF-7, and HCT-116 cells were three human cell lines against which the Plantagoovata ethanol extract was examined. The data in (Table 2) and the findings showed that Plantagoovata ethanol extract has high anticancer activity against all tested cell lines (Figures 2, 3 and 4),

Antioxidant activity

The antioxidant activity of Ethanol extract of Plantagoovata was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. The Ethanol extract of Plantago ovata possessed a relatively strong antioxidant scavenging affinity against DPPH radicals as concluded from their low SC_{50} value as compared with the activity of the standard antioxidant vitamin C (SC_{50} 1.84 µg/ml) (Figures 5,6 and 7).

Using the ideal circumstances mentioned above, Figure 8 displays the overlay of the chosen ion recordings (SIRs) for 16 amino acids. The entire analysis took less than 25 minutes. Retention Time (RT) and SIR windows/values used for each amino acid under study are displayed in Figure 8. With the exception of alanine and cysteine, which each had concentration

ranges of 0.06 to 1.00 µmol/mL, a 5-level calibration set was utilised, encompassing a concentration range of 0.006 to 0.10 µmol/mL.

The typical calibration findings for aspartic acid, glycine, histidine and methiononeare displayed in Figure 8. The fit for all amino acids was quadratic (2nd order). Figure 8 displays the R2 coefficients for the 16 amino acids, with all values more than or equal to 0.999 (n = 3 at each level).

Diabetic assay

Chemical analysis

Creatinine, urea nitrogen, and uric acid were found to be significantly increased in diabetic control rats(+ve), diabetic rats treated with 10% seeds of Plantago ovata showed the best results when compared to control(-ve) as show in **(Table 3) (Figures9&10)**. On the other hand glucose was found to be significantly increased in diabetic control (+ve), diabetic rats treated with extract of 10% seeds of Plantago ovata showed the best results when compared to control (-ve) as show in **(Table 3)[(Figure 9) D].** Lipase was found to be significantly increased in diabetic control (+ve), diabetic rats treated with 1 mm extract of Plantago ovata showed the best results when compared to control (-ve),which inhibited it as show in **(Table 3).**

Histopathological examination

Histopathological changes of the pancreas

Rats in the normal group 1 did not have any histological abnormalities in their pancreas (**Figure 9A**). On the other hand, rats of positive group 2 had pancreatic acini with vacuolar degeneration of the epithelial lining and vacuolation of the islets of Langerhan's cells (**Figure 9B**). Sections from Group 3 treated with 5% Plantago ovata seeds showed no histopathological alterations

in some cases (**Figure 9C**). Additionally, certain sections from group 4 treated with 10% Plantago ovata seeds showed no histopathological alterations (**Figure 9D**). However, rats from group 5 that received 0.5 mL of Plantago ovata seeds did not exhibit any histological alterations in their pancreas [**Figures 9E**]. However, group 6 treated with 1mL sections revealed no histopathological alterations.

Histopathological examination of kidneys

Rats from the control, normal group's kidneys were examined under a microscope to demonstrate the typical histological structure of the renal parenchyma (**Figures 10 A**). Rats in group 2 had kidneys that had glomerular tuft congestion, vacuolation of the epithelial lining of the renal tubules, proteinaceous debris in the tubule lumen, and focal intertubular mononuclear cell infiltration (**Figure 10B**). However, certain renal tubules in the kidneys of group 3 rats showed a minor vacuolation of the epithelial lining (**Figure 10 C**). Some investigated sections from group 5 showed vacuolation of the epithelial lining of some renal tubules and congestion of glomerular tuft but no histological abnormalities (**Figure 10D**). Rats from groups 5 and 6's kidneys, however, did not exhibit any histological alterations [**Figures 10 (E & F)**].

Discussion

GC-MS and the data shown in figure 1 and table 1 were used to describe and identify the chemical components of Plantagoovata's ethanol extract.. These compounds were identified for the first time from Ethanol extract of wild Plantago ovata growing in Libya as; (1,1-Diethoxypropan-2-ol, 7,7-dimethyl-1,3,5-Cycloheptatriene, 5-Methylocta-1,6-dien-3-yne, 2-Ethyl-4-

methyl-1-Pentanol, 2-Ethyl-1-Hexanol, 2-Propyl-1-pentanol, 1,7-Octadiyne, 2-Ethyl-1,4-dimethyl-

Benzene, o-Cymene, Phytol, (Z)-9-Octadecenoic acid, Hexadecanoic acid,2,3-dihydroxypropyl ester, Hexadecadienoic acid, methyl ester, Hexanedioic acid, mono (2-ethylhexyl) ester, Dotriacontane, Diisooctyl phthalate and 7,3',4'-trimethoxy Quercetin. Two of them were discovered to be the primary constituents of this extract. These substances were phytol (25.27%) and 1, 1-Diethoxypropan-2-ol (45.20%).

Antitumor activity of Ethanolextract of Plantago ovata showed that it have a perfect antitumor activity against all tested cell lines, the highest inhibition was for colon HCT-116 as concluded from their IC_{50} value 46 µg / ml , while for HepG2 cells was with low inhibition as concluded from their IC_{50} 74 µg / ml. These good results of antitumor activity with three human cell lines, which are attributed to presence of antitumor compound Phytol with high percent about 25.27% of the chemical constituents of the Ethanolextract of Plantago ovata. .

The antioxidant activity of Ethanol extract of Plantago ovata was founded to have a relatively strong antioxidant scavenging affinity against DPPH radicals as concluded from their low SC_{50} value as compared with the activity of the standard antioxidant vitamin C. Also the same Ethanol extract of Plantago ovata have a relatively strong antioxidant scavenging affinity against DPPH radicals is attributed to presence of antioxidant compound Phytol with high percent about 25.27% of the chemical constituents of the Ethanolextract of Plantago ovata.

The analysis of amino acids plays an important role in a wide range of applications, including those in food, beverage and biomedical industries. Liquid chromatography with pre- or post-column derivatization to boost sensitivity and/or retention of the analytes is a popular technique for amino acid analysis. The LODs for all of the other examined amino acids varied from 0.12 to 1.44 nmol/mL, with the exception of the higher limit for gulatmic acid (caused by its poorer ionisation efficiency). Additionally, all LOD/LOQ ratios were more than five times lower than historical values found for OPA-derivatized amino acids, with the exception of gulatmic acid..

This results elucidate that, Plantago ovata significantly reduced the body weight and food intake **(*An et al., 2021)***.α-amylase ***(Visvanathan et al., 2016)*** and α-glycosidase**(*Bhatia etal., 2019)*** inhibitors could delay the release of glucose or fructose in the small intestine, and subsequently reduce postprandial hyperglycaemia ***(Barber et al., 2021).*** There fore, these inhibitors may be promising oral hypoglycemic agents for preventing type 2 diabetes. As shown in table 3, diabetic rats treated with extract of 10% seeds of Plantago ovata showed the best inhibitory effects on α-amylase and α-glucosidase. The IC_{50} values were determined as 46.00 mg/mL and 54.00 mg/mL. From the above results, we could see that Plantago ovatashowed the best antidiabetic activity.

Conculsion

Seventeen compounds were characterized and identified from Ethanol extract of the Plantago ovata for the first time. Also a good results for both anti-tumor,antioxidant, antidiabetic and antiobesity activities.SoPlantago ovatamay be promising choice to be considered as nutrient and natural drug.

Table- 1: The chemical constituents identified from the Ethanol extract of Plantagoovateby using GC-MS.

Constituents name	Rt	Peak area %
1,1-Diethoxypropan-2-ol	5.17	45.20
7,7-dimethyl-1,3,5-Cycloheptatriene	7.11	9.75
7,7-Dimethyl-1,3,5-Cycloheptatriene	7.21	10.02
5-Methylocta-1,6-dien-3-yne	7.72	0.23
2-Ethyl-4-methyl-1-Pentanol	7.94	0.52
2-Ethyl-1-Hexanol	8.11	0.56
2-Propyl-1-pentanol	8.23	5.06
1,7-Octadiyne	8.43	0.09
2-Ethyl-1,4-dimethyl-Benzene	8.60	2.18
o-Cymene	9.14	0.43
Phytol	31.53	25.27
(Z)-9-Octadecenoic acid	46.69	0.65
Hexadecanoic acid,2,3-dihydroxypropyl ester	48.51	0.62
Hexadecadienoic acid, methyl ester	49.88	1.01
Hexanedioic acid, mono (2-ethylhexyl) ester	50.60	3.51
Dotriacontane	52.47	0.73
Diisooctyl phthalate	53.55	5.47
7,3',4'-trimethoxy Quercetin	56.15	0.43

Rt:Retention time in min.

Table 2: After being treated for 48 hours, the extract had a 50% MTT test maximal inhibitory concentration on the cell viability of colon HCT-116, liver HepG-2, and breast MCF-7 cells. The information is displayed as µg/ml.

Name of cell line	IC_{50} value
MCF7	58 µg / ml
HCT-116	46 µg / ml
HEPG-2	74 µg / ml

Table 3: Effect of seeds of Plantagoovataon kidney functions, Glucoseand enzymes of diabetic rats.

Parameter / Groups	Kidney functions			Glucose	Enzymes		
	Urea	Creatinine	U.A		Lipase	α-Amylase IC_{50} (mg/mL)	α-Glucosidase IC_{50} (mg/mL)
(Group 1) Control –ve	28.10^{e} ±0.110	0.41^{c} ±0.001	1.78^{e} ±0.012	120.01^{e} ±1.011	3.90^{d} ±0.100	49.40^{e} ±0.610	61.01^{c} ±1.010
(Group 2) Control +ve	49.02^{a} ±0.150	0.61^{a} ±0.120	2.92^{a} ±0.110	161.10^{a} ±1.101	5.91^{a} ±0.110	126.00^{a} ±1.000	74.50^{a} ±1. 110
Group 3	40.30^{b} ±0.200	0.54^{b} ±0.002	2.61^{b} ±0.101	141.10^{b} ±1.540	5.60^{b} ±0.200	87.00^{b} ±0.110	69.00^{b} ±1.000
Group 4	34.15^{d} ±0.050	0.41^{c} ±0.003	2.01^{c} ±0.002	114.10^{d} ±2.410	5.15^{c} ±0.250	46.00^{d} ±1.000	54.00^{f} ±1.012
Group 5	36.80^{c} ±0.200	0.46^{bc} ±0.010	2.35^{d} ±0.100	129.10^{c} ±2.400	4.40^{d} ±0.100	66.00^{c} ±0.901	60.00^{d} ±1.010
Group 6	37.10^{c} ±2.65	0.42^{bc} ±0.005	2.01^{c} ±0.011	112.01^{d} ±2.011	4.00^{d} ±0.100	49.50^{f} ±1.500	56.67^{e} ±0.017
LSD	0.33	0.04	0.02	3.49	0.33	-	-
Acarbose	-	-	-	-	-	2.61	0.25

(Mean±SD)

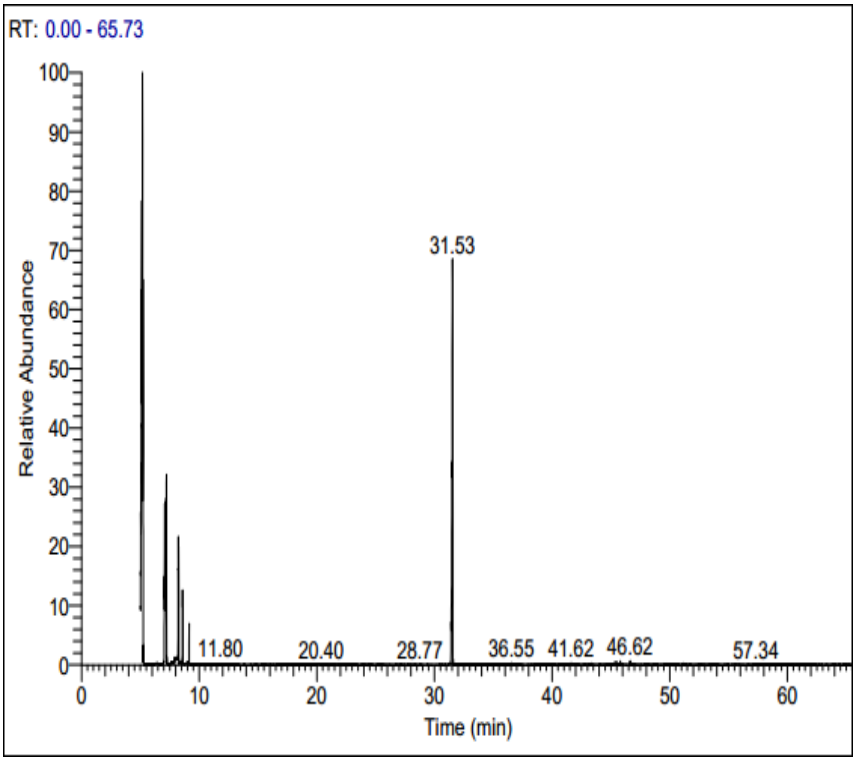


Figure (1)

(GC-MS) of the Ethanol extract ofPlantago ovata.

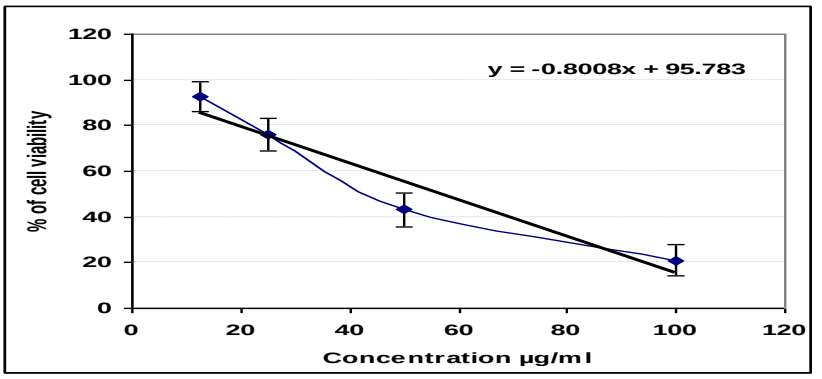


Figure 2

After being exposed to various doses of ethanol extract for 48 hours, liver HepG2 cells had their cell viability assessed using the MTT test. The information is displayed as (Mean ± SE) of µg/ml.

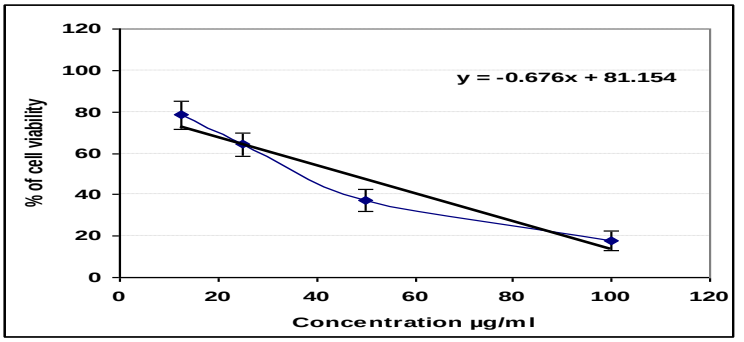


Figure 3

After being exposed to various doses of ethanol extract for 48 hours, colon HCT-116 cells had their cell viability assessed using the MTT test. The information is displayed as (Mean ± SE) of µg/ml.

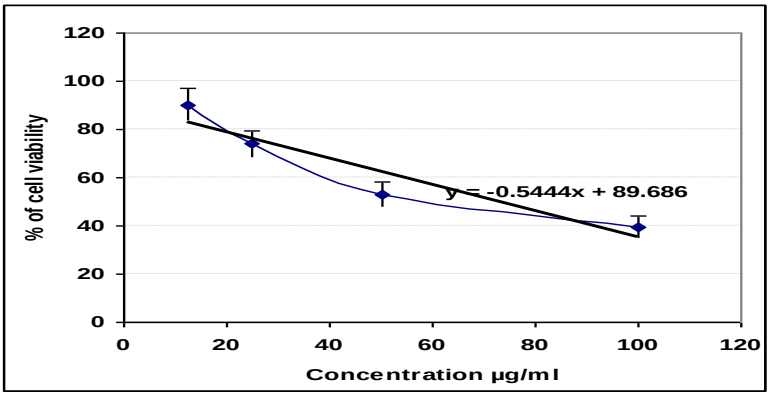


Figure 4

After being exposed to various doses of ethanol extract for 48 hours, breast MCF-7 cells had their cell viability assessed using the MTT test. The information is displayed as (Mean ± SE) of µg/ml.

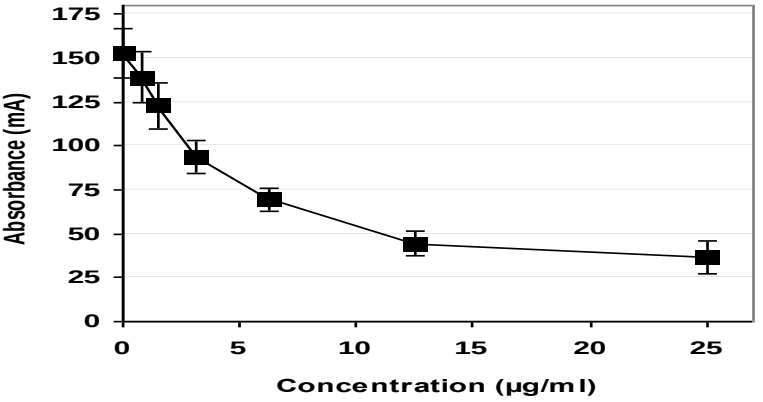


Figure 5

Antioxidant activity of ascorbic acid (vitamin C) against DPPH radicals: SC_{50} 1.84 µg/ml

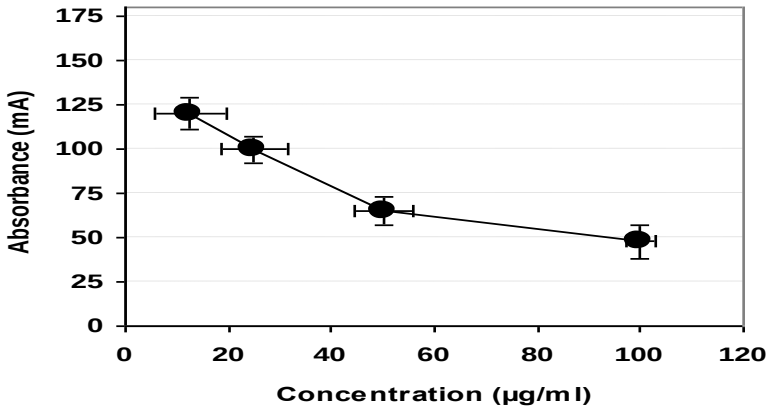


Figure 6

Antioxidant activity of Ethanol extract of Plantago ovataagainst DPPH

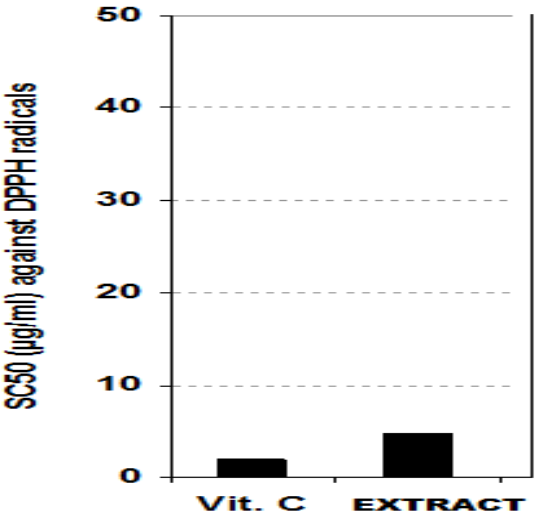
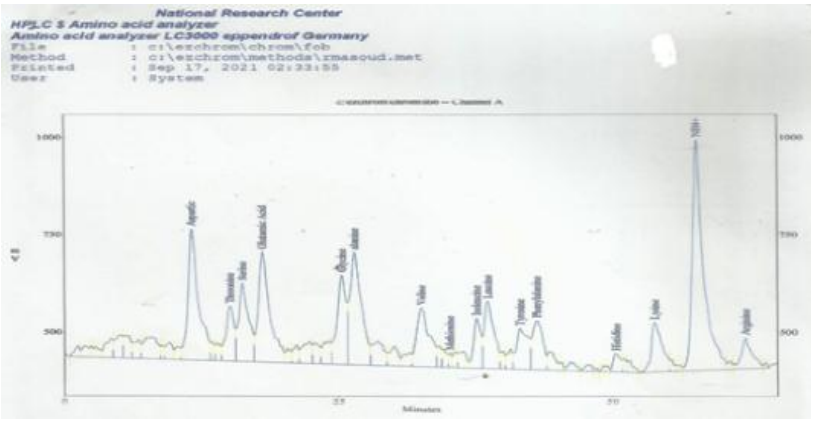


Figure 7

Half maximum scavenging concentration of Ethanol extract of Plantago ovataand vit. C against DPPH radicals. The data are presented as µg/ml.



NO	Name	Retention Time (min)	Concentration (µg/ml)
1	Aspartic	11.57	72.93
2	Threonine	15.07	21.44
3	Serine	10.10	22.22
4	Glutamic acid	10.00	209.92
5	Glycine	25.27	25.94
6	Alanine	26.42	44.06
7	Valine	32.52	27.15
8	Methionine	35.13	2.79
9	Isoleucine	37.55	17.98
10	Leucine	38.58	35.10
11	Tyrosine	41.52	40.57
12	Phenylalanine	43.07	40.34
13	Histidine	50.30	13.20
14	Lysine	53.80	41.76
15	NH4+	57.63	121.61
16	Arginine	62.08	41.97

Figure 8

GC of amino acid in ethanol extract of Plantagoovata.

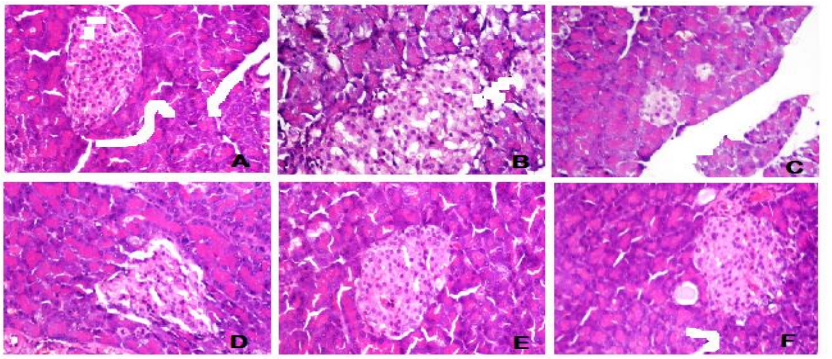


Figure 9:

Effect of seeds of *Plantago ovata* on pancreas section of different groups : **A**) of control rats revealed normal histological; **B**) induced control group **C**) Experimental group treated with 5% seeds of *Plantago ovata* ;**D**) Experimental group treated with 10% seeds of *Plantago ovata*; **E**) Experimental group treated with 0.5 mL extract of seeds of *Plantago ovata*; **F**) Experimental group treated with 1 mL extract of seeds of *Plantago ovata* (H & E X 400).

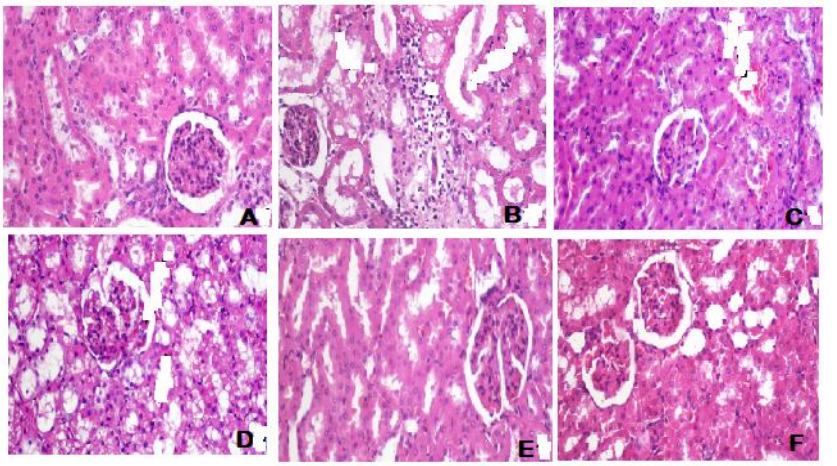


Figure 10:

Effect of seeds of *Plantago ovata* on kidneys section of different groups : **A**) of control rats revealed normal histological; **B**) induced control group **C**) Experimental group treated with 5% seeds of *Plantago ovata* ;**D**) Experimental group treated with 10% seeds of *Plantago ovata*; **E**) Experimental group treated with 0.5 mL extract

of seeds of *Plantago ovata*; **F**) Experimental group treated with 1 mL extract of seeds of *Plantago ovata* (H & E X 400).

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بذور القطونة المصرية الأنشطة الغذائية والبيولوجية والمكونات الكيميائية

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الملخص العربى

تهدف هذه الدراسة إلى تحديد الخصائص المضادة للأكسدة والمضادة للسرطان في بذور القطونة، تم وصف مكوناتها الكيميائية وتحديدها بواسطة GC-MS، تم اختبار مستخلص الإيثانول لبذور القطونة ضد ثلاثة خطوط مختلفة من الخلايا البشرية، بما في ذلك سرطان القولون الغدي (HCT-116)، وسرطان الخلايا الكبدية (HepG-2)، وسرطان الثدي البشري (MCF-7). باستخدام تقنية الكسح الجذري DPPH، تم تقييم النشاط المضاد للأكسدة لمستخلص الإيثانول من بذور القطونة. تم بعد ذلك تقييم قدرة كل عامل مضاد لمرض السكر على تثبيط الأميليز البشري و -جلوكوزيدات، وتمت مقارنة النتائج مع تلك الخاصة بالأدوية المضادة لمرض السكر أكاربوز. بالإضافة إلى ذلك، من المحتمل ان يكون تثبيط الليبيز البنكرياس قد يوضح تأثيرات بذور القطونة. تم تمييز 17 مركبًا من مستخلص الإيثانول لبذور القطونة وتم التعرف عليها بواسطة GC-MS لأول مرة. وعند مقارنتها بفيتامين ج، أظهر النشاط المضاد للأكسدة لمستخلص الإيثانول لبذور القطونة وجود مضادات أكسدة قوية نسبيًا، مما يساعد في القضاء على جذور DPPH. بالإضافة إلى ذلك، فإن خصائص مستخلص الإيثانول لبذور القطونة لها خصائص مضادة للسرطان جيدة ضد جميع خطوط

الخلايا التي تم فحصها. قد تكون الإجراءات المثبطة لبذور القطونة على الجلوكوزيدات البشرية والأميليزمفيدة في تنظيم نسبة السكر في الدم بعد الأكل وامتصاص السكر دون عيوب العلاج بالأكاربوز.

الكلمات الدالة: بذور القطونة، نشاط مضاد للسرطان، نشاط مضاد للأكسدة، نشاط مضاد لمرض السكر.