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Full Length Article

[](http://crossmark.crossref.org/dialog/?doi=10.1016/j.ejbas.2018.11.001&domain=pdf)Anti-cancer potential of the lipoidal and flavonoidal compounds from

*Pisum sativum* and *Vicia faba* peels

Amal M. El-Feky [⇑](#_bookmark0), Marwa M. Elbatanony, Marwa M. Mounier

*Pharmacognosy Department, National Research Centre, 33 El Buhouth St., Dokki, Giza, P.O.12622 (ID: 60014618), Egypt*

# a r t i c l e i n f o

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Anti- cancer

# a b s t r a c t

For a long time, plant secondary metabolites have been strongly examined for their antitumor and cytotoxic impacts. These days, there is another pattern of making utilization of the waste products of plants because of their extravagance of numerous phytochemical components and adequacy on human wellbeing. This research work is handling the effect of diversity of lipoidal and phenolic compounds found in the peels of two common edible plants in the Middle East; *Pisum sativum* and *Vicia faba* L. for their assesment as anticancer agents. The GC/MS of the *n*-hexane extract of both plant peels led to identification of twenty compounds (82.99%) and seventeen compounds (85.97%) of the total lipoidal contents from *P. sativum* and *V. faba*, respectively. While the HPLC analysis of the ethyl acetate fraction of the two plant peels resulted in recognition of 17 flavonoids and 18 phenolics from *P. sativum* and 16 flavonoids and 17 phenolics from *V. faba*. Moreover, four flavonoidal compounds were isolated to our knowledge for the first time from the peels and tested separately against different human cancer cell lines and the mode of action of the most potent compound has been determined. *P. sativum* ethyl acetate fraction possessed the highest scavenging activity (31.2%) as well as the most cytotoxic effect on breast carcinoma cell line. Apigenin proved to be the most potent tested compound on (MCF-7) and has no cytotoxic effect on normal human skin cell lines.

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1. Introduction

Peels of vegetables and fruits are frequently tossed or used to feed the livestock and as fertilizers. These wastes are profoundly inclined to microbial spoilage and thusly create significant issues to the environment. So that, these wastes ought to be figured out how to be used advantageously. Nowadays, many studies are performed to utilize these wastes to reduce the environmental pollution and get some medicinal benefits [[1]](#_bookmark17). Where fruit peels are an important source of bioactive compounds mainly as anti- oxidant and anticancer agents against colon, prostate and breast cancers [[2]](#_bookmark18). Therefore, it is necessary to unveil the biological activities of these peels and take the benefits from their waste materials, in addition to investigate their chemical composition to encourage adequate reuse of these wastes for several applica- tions in the medicine [[3]](#_bookmark19).

It has turned out to be certain that tumor is the principle driving reason for death in developed countries as well as developing countries. Mankind has been struggling with great efforts to get improved and discover cheaper treatments with fewer drawbacks

\* Corresponding author.

*E-mail address:* [ammelfeky@hotmail.com](mailto:ammelfeky@hotmail.com) (A.M. El-Feky).

to decrease the commonness of this disease and its resulting mor- tality. Legumes are well known of being rich in many bioactive non-nutrient components (phytochemicals) alongside their nutri- tional valued compounds (protein, carbohydrates, dietary fibers, and vitamins). Both plants under study belong to family Fabaceae. *Pisum sativum* L. is commonly known as the green pea or garden pea. In 2002, Troszynhska et al. [[4]](#_bookmark22) proved that the acetone extract of the seed coat has antibacterial, antidiabetic, antifungal, anti-inflammatory, antihypercholesterolemic, antioxidant and anticancer properties. The HPLC analysis of the phenolic com- pounds in the same study showed the presence of some phenolic acids (benzoic, cinnamic acids, and their derivatives as well as some flavonoids (apigenin-7-glucoside, quercetin-3-rhamnoside, kaempferol-3- glucoside as well as other flavonoids). On the other hand, *Vicia faba* L. is among the oldest plant in the world [[5]](#_bookmark23), and considered an essential source of protein and energy as it is rich in a large amount of amino acids [[6]](#_bookmark24), also a potent source of levodopa; a precursor of dopamine, so that, it can be used for the treatment of Parkinson’s disease [[5]](#_bookmark23).

The present study focused on phytochemical evaluation of lipoi- dal and phenolic extracts in the peels of *Pisum sativum* L. and *Vicia faba* L. for their assesment as anticancer agents.

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1. Materials and methods
   1. *Material for phytochemical study*
      1. *Plant material*

The fresh fruits of both *Pisum sativum* and *Vicia faba* were col- lected from local markets in Cairo, Egypt in February 2016. Each species was peeled off; the peels were dried in shades, then grinded and kept in sealed bags.

* + 1. *Preparation of the extracts*

The dried powdered peels of *P. sativum* and *V. faba* were sepa- rately defatted with *n*-hexane then extracted with ethyl acetate several times.

The obtained four extracts were concentrated under reduced pressure at 45 °C using the rotary evaporator to 1/10th of the initial volume and kept in refrigerator for the further studies.

* + 1. *Chemicals and reagents*

All the chemicals and reagents were of analytical grade and pur- chased from Merck.

* + 1. *GC/MS analysis of the lipoidal extracts*

The n-hexane extract of both plant peels were analyzed using GC/MS technique for identification of sterols, terpenes and fatty acid methyl esters.

* + 1. *HPLC determination of phenolics and flavonoids*

High-performance liquid chromatography (HPLC) using Agilent Technologies 1100 series liquid chromatograph coupled with an auto sampler and a diode-array detector was performed for the identification and quantification of flavonoids and phenolics in the ethyl acetate fraction of *P. sativum* and *V. faba* according to

[[7] and [8]](#_bookmark25).

* + 1. *Screening of phenolics and flavonoids*

The initial screening for the ethyl acetate fractions of both plants was carried out separately with the basic qualitative test for flavonoids, where 0.5 ml of the extract was mixed with 2 ml of conc. H2SO4 and few magnesium turnings. Further, Thin Layer Chromatography (TLC) of the fraction was carried out using the solvent system chloroform–methanol (90: 10 v/v). In the TLC screening procedure, a thin strip of 3x10 cm of TLC Silica Plate (Sil- ica gel 60 F254, Merck), was taken and impregnated with the fine drop of fraction. The plate was then air dried and kept for the development in chromatographic chamber containing10ml of the prepared solvent system. After the successful development, the plate was examined under the UVChamber at 254 and 366 nm. The presence of flavonoid constituents was confirmed by spraying with 1% ethanolic solution of AlCl3 [[9]](#_bookmark26).

* + 1. *Isolation and purification of flavonoids*

The developing of the ethyl acetate fractions of both species was carried out separately on preparative TLC using Chloroform– methanol (90:10 v/v) as developing system. The plates were exam- ined under the UV light at 254 and 366 nm, resp. [[10] and [11]](#_bookmark10), and subjected to AlCl3 solution. The selected bands marked and scratched then collected. The R*f* values of the isolated compounds were recorded and co-chromatographed against the available authentic flavonoids for the confirmation of the isolated com- pounds at the same R*f*. the isolated compounds were identified by different spectral analyses (UV, H1-NMR, IR and MS spectrometry).

Determination of melting point and different Spectral Analyses: Koffler’s heating stage microscope was used to determine the

melting point, UV–Visible Spectrophotometer double beam UVD– 3500 spectrophotometer, Labomed, Inc., Visible Spectrophotome- ter, Shimadzu UV 240 (PIN 204-58000) (Japane), Infrared spec- trophotometer, Perkin-Elmer 283 (Germany), Nuclear Magnetic Resonance spectrometers JEOL EX-270 MHz, 300 MHz and 500 MHz for determination of H1-NMR, Mass spectrometer; Finni- gan Model 3200 at 70 eV.

* 1. *Material for antioxidant and cytotoxic study*
     1. *Free radical scavenging activity*

DPPH●. The absorbance was measured at 517 nm, after 30 min The four extracts were screened at 50 lg/ml using 0.1 mM incubation [[12]](#_bookmark10). Ascorbic acid was used as standard reference [[13]](#_bookmark10).

* + 1. *Chemicals*

DPPH● was obtained from Fluka. Vitamin C (ascorbic acid) obtained from Laboratory Rasayan. Methanol used was of analyti-

cal grade.

* + 1. *Cell lines*

Human breast carcinoma (MCF-7 cell line) and colon carcinoma (HCT-116 cell line) were obtained from Karolinska Center, Depart- ment of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden.

* + 1. *Determination of LC50 values*

It was performed using SPSS computer program (SPSS for win- dows, statistical analysis software package/version 9/1989 SPSS Inc., Chicago, USA).

* + 1. *Cell culture*

The procedure was done in laminar air flow cabinet biosafety class II level. Culturing and subculturing were carried out accord- ing to Thabrew et al. [[14]](#_bookmark10). Doxorubicin was used as a positive con- trol. DMSO used as negative control.

* + 1. *Cell viability assay*

This was done according to Mosmann et al. [[15]](#_bookmark10). The cells were

20 × 103 cells/well in case of HCT-116 cell lines using 96-well seeded at concentration of 10x103cells per well in case of MCF-7, plates at 37 °C. After 48 h’ incubation, the medium was aspirated

and 40 ll MTT salt (2.5 mg/ml) were added and furtherincubated for 4 h. 200 ll 10% sodium dodecyl sulphate (SDS) was added. The absorbance was measured at 595 nm.

* + 1. *Measurement of Bcl-2 levels*

BCL-2 in the samples and standards were estimated according to [[16]](#_bookmark10). A biotin-conjugated antibody was added followed by streptavidin-HRP. The reaction is then terminated by addition of acid and absorbance was measured at 450 nm.

* + 1. *Measurement of Bax levels*

Bax protein level were evaluated according to [[17]](#_bookmark10). Monoclonal antibody specific to Bax captured on the plateis added. After incu- bation, Streptavidin conjugated to Horseradish peroxidase is added. The reaction is terminated by the addition of acid and opti- cal density of the color produced measured at 450 nm.

* + 1. *Human CASP7 (Caspase 7) estimation*

The micro ELISA plate provided in this kit pre-coated with CASP7 specific antibody. A biotinylated CASP7antibodyand Avidin-Horseradish Peroxidase (HRP) conjugate was added. Aspire the excess components. The substrate solution was added. wells that contain CASP7, biotinylated detection antibody and Avidin- HRP conjugate will appear blue in color. The color turns yellowfol-

lowed the addition of sulphuric acid solution. The optical density (OD) was measured at a wavelength of 450 nm ± 2 nm. [[18]](#_bookmark10).

1. Results
   1. *GC/MS analysis of the lipoidal extracts*

GC/MS analysis of the *n-*hexane extracts of *P. sativum* and *V. faba* was performed, and identification of the constituents was car- ried out by comparison of their spectral fragmentation patterns with those of the available database libraries Wiley (Wiley Int.) USA and NIST (Nat. Inst. St. Technol., USA)] and/or published data in Adams [[19]](#_bookmark10) using Aglient 6890, 70 eV with positive ion mode. Quantitative determination was carried out based on peak area integration. The identified components are compiled in [Tables 1](#_bookmark2) [and 2](#_bookmark2).Twenty compounds were identified from the lipoidal matter of *P. sativum*, representing 82.99% of the total content from which 2, 2-dimethyl-1-(2,4,6 trimethylphenyl) was the major compound (12.56%) followed by 6-Phenylundecane (12.18%). Nevertheless, seventeen compounds were identified from *V. faba* representing 85.97% of the total lipoidal content, 5-phenylundecane was identi- fied as the main compound of *V. faba* (23.24%) followed by 2 (Ben- zoyloxy) cycloheptanone (12.74%).

* 1. *Identification and quantification of phenolics and flavonoids*

[Tables 3 and 4](#_bookmark1) summarize the results of HPLC analysis of the ethyl acetate fraction of both plant peels. The total identified flavo- noids from *P. Sativum* were 17 compounds representing 19.31 mg/

Table 2

GC/MS analysis of the *n-*hexane extract of *V. faba*.

|  |  |  |  |
| --- | --- | --- | --- |
| Compound | Mol. Weight | BP | Relative area % |
| n-Decane | 142 | 57 | 0.68 |
| Nonanal | 142 | 41 | 3.67 |
| Decanol | 158 | 41 | 1.12 |
| Dodecanol | 186 | 41 | 0.74 |
| 3-Methyl pentenyl phenyl ketone | 188 | 161 | 2.61 |
| 7-phenyl Tridecane | 218 | 91 | 6.03 |
| Pentadecanol | 228 | 41 | 3.91 |
| 5-phenylundecane | 232 | 91 | 23.24 |
| 2 (Benzoyloxy)cycloheptanone | 232 | 105 | 12.74 |
| n-Heptadecane | 240 | 57 | 2.61 |
| Hexadecanol | 242 | 41 | 3.65 |
| 7,7-Diphenyl-2,4,6-heptatrienal | 260 | 260 | 8.91 |
| Methyl palmitate | 270 | 74 | 6.24 |
| n-Eicosene | 280 | 41 | 2.24 |
| Methyl linoleate | 294 | 67 | 5.56 |
| n-Heneicosane | 296 | 57 | 0.90 |
| a, a-Carotene4,4'-dione | 564 | 83 | 1.12 |
| Hydrocarbons |  |  | 35.70 |
| Fatty alcohols |  |  | 9.42 |
| Aldehydes |  |  | 12.58 |
| Ketones |  |  | 16.47 |
| Esters |  |  | 11.80 |
| Total identified |  |  | 85.97 |

Table 3

HPLC analysis of flavonoids in the ethyl acetate fractions of *P. sativum* and *V. faba*.

Flavonoids Concentration (mg/100 g)

*Pisum sativum Vicia faba*

|  |  |  |  |
| --- | --- | --- | --- |
| g of the total content, while 16 compounds were identified for *V.*  *faba* representing 12.30 mg/g dry weight of the whole fraction. | Apigenin-6-arabinose-8-galactose Apigenin-6-rhamnose-8-glucose | 170.01  357.13 | 68.46  185.53 |
| Hesperidin was found to be the major flavonoid in both plants | *Naringin* | 201.41 | 109.79 |
| recording (6.10 mg/g) for *P. sativum* and (2.46 mg/g)for *V. faba*. | *Hesperidin* | 605.94 | 245.85 |

On the other hand, regarding the results of total identified pheno- lics, 18 compounds were identified from *P. sativum* (89.65 mg/g) of dry weight of which pyrogallol (8.61 mg/g) was the main phenolic followed by catechol (8.53 mg/g). Moreover, 17 phenolic com- pounds were identified from *V.faba* and the total phenolic content

Table 1

GC/MS analysis of the *n-*hexane extract of *P. sativum*:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | *Kaempferol* | 019.79 | 016.61 |
| Compound | Mol. | BP | Relative |  | Rhamentin | 248.32 | 070.12 |
|  | Weight |  | area % |  | Apigenin | 014.31 | 056.41 |
| n-Dodecane | 170 | 43 | 3.61 |  |  |  |  |
| n-Tridecane | 184 | 43 | 4.75 |  |  |  |  |

*Rutin* 83.01 030.24

Apigenin-7-O-neohespiroside 104.13 027.30

*Kaempferol-*3,7-dirhamnoside 013.26 008.20

*Quercetrin* 256.26 –

Apigenin-7-glucose 147.59 015.48

*Acacetin-7-neo hesperside* 136.87 043.28

*Acacetin neo rutinoside* 061.77 073.71

*Quercetin* 056.90 095.20

|  |  |  |
| --- | --- | --- |
| *Naringenin* | 024.59 | 071.98 |
| *Hespirtin* | 158.29 | 112.28 |

recorded (62.40 mg/g). Pyrogallol also was the main compound identified (8.50 mg/g) followed by q-hydroxy benzoic acid (8.10 mg/g). These recordable numbers of phenolics with reason- able amounts were very encouraging for the cytotoxic study.

|  |  |  |  |
| --- | --- | --- | --- |
| Dodecanol | 186 | 41 | 2.31 |
| 2,2dimethyl-1-(2,4,6trimethylphenyl) Propan- 1- | 204 | 147 | 12.56 |
| one  Di-t-butylphenol | 206 | 191 | 4.35 |
| Tetradecanol | 214 | 41 | 1.77 |
| (4E,8E)5-Propyl trideca-4,8-dien-6-yne | 218 | 91 | 2.77 |
| 2-Phenyl Decane | 218 | 105 | 3.89 |
| 6-Phenylundecane | 232 | 91 | 12.18 |
| 6-Phenyldodecane | 246 | 91 | 10.63 |
| n-Hexadecanoic acid | 256 | 43 | 10.35 |
| 6-Phenyl tridecane | 260 | 91 | 7.12 |
| 2- Phenyl tridecane | 260 | 105 | 0.81 |
| 8,9-Dihydrocyclohept phenalen-7,10-dione | 260 | 91 | 0.86 |
| Octadecanol | 270 | 41 | 2.80 |
| n-Docosane | 310 | 43 | 0.28 |

* 1. *Structure characterization of the isolated phenolics from P. Sativum*

*Apigenin*: Yellow colored amorphous powder, melting point: 180–181 °C, UV: deep purple, UV/NH3: yellow green. R*f* 0.87 using Chloroform–methanol (90:10 v/v) as developing system, UV k

MeOH max (nm) 267, 296 sh, 336; +NaOMe 275, 324, 392; +AlCl

3

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Pregn-4-ene-3,20-dione | 314 | 124 | 0.20 | 276, 301, 348, 384; +AlCl3 – HCl 276, 299, 340, 381 nm; + NaOAc |
| 9-n- hexyl heptadecane | 324 | 43 | 0.28 | 274, 301, 376; +NaOAc – H3BO3 268, 303sh, 338. IR data showed |

Tetracosane 338 57 0.46

4-Phenyl Eicosane 358 91 1.01

Hydrocarbons 47.79

Fatty alcohols 11.23

Ketones 13.62

Acids 10.35

Total identified 82.99

a broad intermolecular OH stretch vibrations band at 3333 cm—1, an aromatic CAH stretch appeared at 3040 cm—1, in addition to a vibration band at 1646 cm—1 characteristic for flavone of conjuga- 1801 cm—1 for lactone ring, in addition to three vibration bands at (1466, 1497, and 1578 cm—1) for the ring C@C, while tion between the C@O and double bonded of C2–C3, also,

Table 4

HPLC analysis of phenolics in the ethyl acetate fractions of *P. sativum* and *V. faba*.

Phenolic compounds Concentration (ppm)

*Pisum sativum Vicia faba*

Gallic acid 218.45 15.44

Pyrogallol 860.06 850.38

co-chromatography against authentic and comparing with the published data [[22]](#_bookmark13), this compound was identified as apigenin-7- O-b-D-glucopyranoside.

* 1. *Structure characterization of the isolated phenolics from V. faba Quercetin:* It was in the form of yellow amorphous powder,

melting point: 315–316 °C, UV: yellow, UV/ NH : yellow, R 0.55,

|  |  |  |
| --- | --- | --- |
| 4-Amino benzoic acid | 069.25 | 012.63 |
| Protocatchuic acid | 426.15 | 400.36 |
| Catechein | 795.74 | 599.65 |

3 *f*

|  |  |  |  |
| --- | --- | --- | --- |
| Chlorogenic acid | 742.28 | 197.24 | UV data k MeOH max (nm) 255, 269 sh, 301 sh, 370; +NaOMe |
| Catechol | 852.94 | 158.36 | 247, 321; +AlCl3 272, 304 sh, 333, 458; +AlCl3 – HCl 265, 301 sh, |
| Caffeine | 656.52 | 194.47 | 359, 428 nm; +NaOAc 257 sh, 274, 329, 390; +NaOAc – H3BO3 |

q-Hydroxy benzoic acid 588.94 810.02

Caffeic acid 146.11 106.54

Vanillic acid 536.67 –

q-Coumaric acid 462.93 150.76

Ferulic acid 788.29 609.38

Iso-Ferulic acid 210.39 076.25

Ellagic acid 433.87 –

Alpha coumaric acid – 066.95

Benzoic acid 492.22 737.22

3,4,5-Methoxycinnamic acid 477.43 213.31

Cinnamic acid 206.97 219.50

1466 cm—1 denotes the characteristic of CAOAH stretch. The inten-

sive band at 1024 cm—1 was most probably the result of CAOAC

261, 303sh, 388.IR data: at 3421.20 cm—1 for (AOH group), 2932.54 cm—1 representing (CH-stretching), 1067.25 cm—1 (CAO bond), peaks at 1612.0, 1561.0, 1421.6 cm—1 significant for (aro- matic ring system), H1-NMR (400 MHz, CH3OH):H-8 appeared at

d 6.47 (d, J = 1.1, 1H) and H-6 at d 6.21 (d, J = 1.5, 1H), H-2' at d

7.74 (d, J = 2.2, 1H-6'), H-5' showed at d 6.95 (d, *J* = 8.4, 1H-6'), H-

6' at 7.64 (d,d., J = 2.2, 1H-2'-*J* = 8.4, 1H-5), *Mass Spectrum* illus-

trated the [M] + at 302 (100%) for molecular formula C15H10O7,

beside other main fragments at *m*/*z* with relative abundance: 301 (60%), 151 (58%).

*Quercetrin* (Quercetin-3-rhamnoside): Yellow powder, melting

point: 181–182 °C, gave deep purple color under UV and yellow green with UV/NH , *R* 0.60, UV data k MeOH max (nm) 256,

3 *f*

stretch from the central heterocyclic ring. H1-NMR (400 MHz,

CH3OH):d7.75 (2H,d, J = 8.3 Hz, H-2' and H-6'),6.86 (2H,d,

J = 8.3 Hz, H-3 and H-5'), 6.79 (1H, d, J = 2.1 Hz, H-6), 6.68 (1H, d,

J = 2.1 Hz, H-8), 6.65 (1H, s, H-3).

*Mass spectrum* confirmed the molecular formula of C15H10O5, EI- MS *m*/*z*: [M + H]+ 271, the base peak was observed at *m*/*z* 117, another significant peak at *m*/*z* 151 (31%). Apigenin can undergo sequential loss of C2H2O and CO2to give a fragment at *m*/*z* 183.

*Apigenin-7-O-glucoside:* Yellow crystal, melting point: 227 °C

(223–226 °C) [[20]](#_bookmark11), UV: dark purple, UV/NH3: yellow green. R*f* 0.89, UVdata k MeOH max (nm) 267, 334; +NaOMe244sh, 267,

300, 386; +AlCl3 276, 302, 349, 383; +AlCl3 – HCl278, 303, 343,

380 nm; +NaOAc 270, 350, 388; +NaOAc – H3BO3 270, 343.IR data:

1462, 1501, and 1580 cm—1 for the ring C@C, while 1466 cm—1 CH3OH): d ppm 7.81 (2H, d, J = 9.1 Hz, H-2'/6'), 7.36 (2H, d, denotes the characteristic of CAOAH stretch, H1-NMR (400 MHz,

J = 9.1 Hz, H-3'/5'), 6.65 (1H, s, H-3), 6.55 (1H, d, J = 2.6 Hz, H-6),

6.88 (1H, d, J = 2.6 Hz, H-8), d 5.01 (1H, d, J = 7 Hz, H-1''). *Mass spec-*

*trum* gave M+at 433 for molecular formula C21H20O10 other

fragments observed at 443 (48%), 473 (42%) and 503 (22%) especially indicate the presence of substituted pentose. From the spectral analyses, melting point [[21]](#_bookmark12) (Neil et al., 2001),

265sh, 301 sh, 350; + NaOMe 270, 326, 393; +AlCl3 276, 304sh,

333, 430; +AlCl3 – HCl 272, 303sh, 353, 401 nm; +NaOAc 272,

3425.00 cm—1 broad represents hydroxyl group, (AOH stretch), peak at 2980.02 cm—1 represents CAH stretching (ACH), peak at 322, 372; +NaOAc – H3BO3260, 300sh, 367. *IR analysis*: peak at 1371.50 cm—1 represents CH3bending and peak at 1465.13 cm—1 1720 cm—1 to 1732 cm represents conjugated ketone (C@O), peak at 1430.00 cm—1 and 653.94 cm—1 represent aromatic ring system, represents bending of methylene and methyl group, peak between peak at 1300.21 cm—1 C@C group, peak at 1078.21 cm—1 represents etherlink (CAOACA). 1H-NMR (400 MHz, CH3OH) d: 7.51 (1H, d,

J = 1.4 Hz, H-2'), 7.61 (1H, dd, J = 8.3, 1.8 Hz, H-6'), 6.65 (1H, d,

J = 8.1 Hz, H-5'), 6.32 (1H, d, J = 1.9 Hz, H-8), 6.20 (1H, d,

J = 1.7 Hz, H-6), 5.10 (1H, d, J = 2.2 Hz, H-1''), 4.25–3.20 (sugar H),

0.99 (3H, d, J = 6.0 Hz, H-6''). *Mass spectrum* was confirmed the

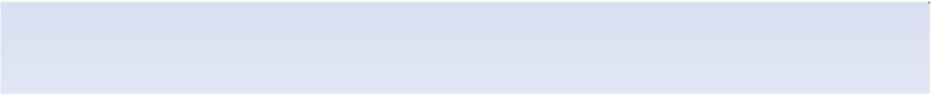
molecular formula of C21H20O11 [M+1] at *m*/*z* 448 (100%), in addi-

[M-H-C15H16O9]—. tion to another significant fragment was noticed at 107 (30%) for

It is to be noted that the forementioned flavonoidal compounds

were isolated to our knowledge for the first time from these peels, while some of them or their derivatives were previously isolated from some edible parts [[23]](#_bookmark14).

Fig. 1. The antioxidant activities of the four extracts, *in vitro*, using the DPPH● assay.



**DPPH**

35

30

25

20

15

10

5

0

DPPH

P.sativum n- P.sativum V.faba n-hexane V.faba Eth. hexaneextract Eth.acetate Fr. extract Acetate Fr.

1. Results of antioxidant and cytotoxic study
   1. *Free radical scavenging activity*

The four extracts showed weak scavenging activity of DPPH● where *P. sativum* ethyl acetate fraction possessed the highest scav-

enging activity (31.2%) followed by *V. faba* ethyl acetate fraction (9.8%) as showed in [Fig. 1](#_bookmark3).

* 1. *Cytotoxic study of the four extracts*

The four extracts were preliminary screening at 100 ppm for their antiproliferative effect using two human tumor cell lines [hu- man breast carcinoma (MCF-7), human colon carcinoma (HCT- 116)]. *P. sativum* ethyl acetate fraction showed high activity over breast cancer cell line with 73.6% and low activity over colon tumor cell line (HCT-116) with 21% while *P. sativum n*-hexane extract exhibited 26.1, 28.3 on both cell lines, resp. On the other hand *V. faba n*-hexane extract gave 40.2 and 6.7, while *V. faba* ethyl acetate fraction 32.3, 22.1 over both MCF-7 and HCT-116, respec- tively, [Fig. 2](#_bookmark4). The *P. sativum* ethyl acetate fraction which possessed the highest activity over breast carcinoma cell line was further

assayed at lower concentrations to calculate its LC50 which was calculated as 73.4 ± 1.7 on Breast carcinoma cell line.

1. Cytotoxic study of the isolated compounds

The four compounds were tested for their cytotoxic activity over [(MCF-7) and (HCT-116)] as shown in [Fig. 3](#_bookmark5). Apigenin pos- sessed promising cytotoxic effect on breast cancer and colon cell line, so it is further screened over the cell lines to calculate its LC50 value where it recorded the following LC50 values; 44.8, 60.8 over breast and colon tumor cell line, respectively. Apigenin proved to be the most potent tested compound on (MCF-7) and has no cytotoxic effect on normal human skin cell lines, so it is directed to explore its apoptotic mode of action.

1. Apoptotic mechanism of Apigenin

Apoptosis, or programmed cell death, takes place in all living organisms. Disruption of apoptotic mechanisms could lead to the deregulation of cell proliferation. Targeting the process of apoptosis is an appropriate strategy for prevention and treatment of cancer. Based upon that apigenin effectively suppressed the growth of both



80

70

60

50

40

30

20

10

0

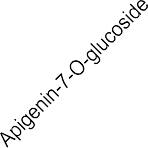
MCF-7

HCT-116

P.sativum hexane P.sativum V. Fabahexane V. Faba

ethylacetate ethylacetate

Fig. 2. Screening of the four extracts on breast carcinoma (MCF-7) and colon carcinoma (HCT-116).



100

90

80

70

60

50

40

30

20

10

0

MCF-7

HCT-116

Fig. 3. Screening of the 4 compounds on breast carcinoma (MCF-7) and colon carcinoma (HCT-116), the results expressed by % of inhibition at 100 ug/ml.

colon and breast tumor cell line with LC50 on breast less than colon, changes in expression of apoptosis- (apoptosis-related genes) of apigenin over (MCF-7) were investigated. Quantitative estimation of BcL2, Bax, Bax/ Bcl2 ratio and caspase 7 were determined. Bcl-2 family members are associated with the intrinsic pathway of pop- tosis comprised of both pro-apoptotic (e.g. Bax, Bid) and anti- apoptotic (e.g. Bcl-2, Bcl-XL) members. Expression of the anti- apoptotic Bcl-2 in breast cancer cell line treated with apigenin was significantly decreased as compared with control ‘‘untreated



**Caspase 7**

2

1.8

1.6

1.4

1.2

1

0.8

0.6

0.4

0.2

0

Caspase 7

Apigenin Doxorubucin Control

Fig. 4. BcL 2 quantitative analysis, where Y axis represents The protein levels (ng/ml).



**BcL2**

6

5

4

3

2

1

0

BcL2

Apigenin Doxorubucin Control



**BAX**

300

250

200

150

100

50

0

BAX

Apigenin Doxorubucin Control

Fig. 5. Bax quantitative analysis, where Y axis represents the protein levels (ng/ml).

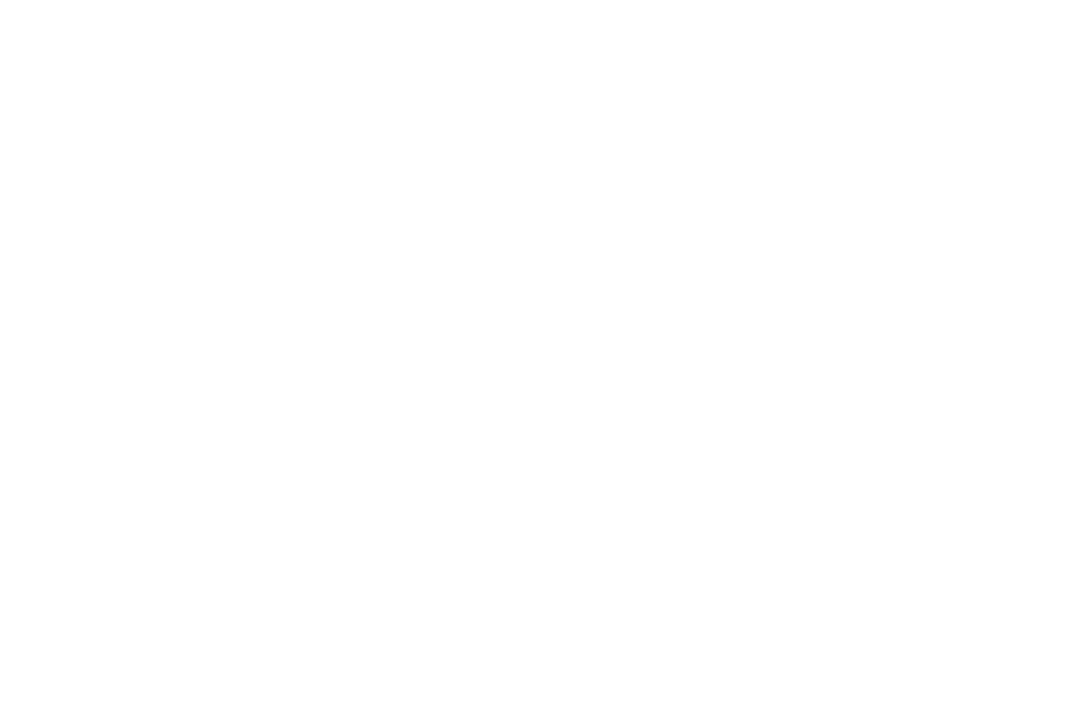
Fig. 7. Caspase 7 protein level (ng/ml) in treated breast cancer cells and untreated compared to doxorubicin.

breast cancer cells ‘‘as shown in [Fig. 4](#_bookmark6). Apigenin resulted in an increase in Bax protein levels in MCF-7 cells chart [Fig. 5](#_bookmark8).

The ratio between pro- and anti-apoptotic levels an important determinant of cell survival. The Bax/Bcl-2 ratio in cells treated with apigenin increased drastically, indicating that apoptosis induced in breast cancer cells might be mediated by the mitochon- drial pathway ([Fig. 6](#_bookmark9)). Caspase-7 is a member of the caspase family of proteins plays a central role in the apoptotic machinery. Api- genin showed increased in caspase 7 levels in treated breast cancer cell line in comparison to untreated breast cancer cell line as shown in [Fig. 7](#_bookmark7).

1. Discussion

In our work we attempted to discover the correlation of the lipoidal and phenolic compounds and their impact on some human cell line carcinoma. An extensive number of hydrocarbons and triterpenoids with different groups are known to exhibit chemo- prevention and cytotoxicity against many tumor cells as well as anticancer remedy both *in vitro* and *in vivo* [[24–26]](#_bookmark15). Phenolics with its assorted variety of classes are famous of inducing apoptosis and cytotoxic activities on various cancer cell lines. The ability of scav- enging of radicals and antioxidant properties are principally responsible for the antitumor activities of phenolic compounds.



**Bax/Bcl -2 ratio**

3.5

3

2.5

2

1.5

1

0.5

0

Bax/Bcl-2 ratio

Apigenin Doxorubucin Control

Fig. 6. Bax-Bcl2 ratio.

Quantitative structure–activity relationship studies on the cyto- toxic effect of phenolic compounds have been examined in the recent period by many studies [[27–29]](#_bookmark16).

Hesperidin which is a major flavonoid in the ethyl acetate frac- tions of both plants under study, has demonstrated to protectively affect CCl4-induced oxidative stress and resultant dysfunction of rat liver which has been correlated to its antioxidant property [[30]](#_bookmark20). Another study investigated hesperidin impact on the prolifer- ation of MCF-7 human breast cancer cells, and prostate cancer cells [[31]](#_bookmark21). On the other hand, pyrogallol, a major phenolic compound identified from *V. faba* exhibited a moderate effect against prostate cancer cell in a previous study Chew et al. [[32]](#_bookmark27) Catechol which is another significant phenolic identified from *P. sativum* apparently

turned out to have a sensible effect on two breast cancer cell lines (MCF-7 and MDA-MB-231[[33]](#_bookmark28). q-Hydroxy benzoic acid, a phenolic acid present in *P. sativum* and *V. faba* with great amounts was pre- viously proven to have strong cytotoxic activity on both colon (HCT116) and liver (HEPG2) carcinoma cell line [[34]](#_bookmark29). While pyro- gallol exhibited a potential anticancer activities against renal cell carcinoma cell lines and no activity was observed with normal healthy cells [[35]](#_bookmark30).

1. Conclusion

In the present investigation, it can be clearly noticed that both plant peels are wealthy in phenolics and flavonoids which assumed an important role as antioxidant and anticancer as well. Our out- comes affirmed that apigenin isolated from *P. sativum* ethyl acetate fraction caused the loss of cell viability of MCF-7 breast cancer cells and induces mitochondrial-dependent apoptosis through Bax acti- vation, Bcl-2 down regulation and imbalance between *Bcl-2* and *Bax* expressions which prompt mitochondria-mediated caspases pathways including activation of caspase-7. These results urge to make the best utilization of plant wastes and reconsider their advantageous use as a promising and effective remedy against many cancer diseases.

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